BREEDING AND ORGANOLEPTIC STUDIES OF HIGH-SUCROSE AND HIGH LYSINE MUTANTS IN MAIZE (*ZEA MAYS* L.)

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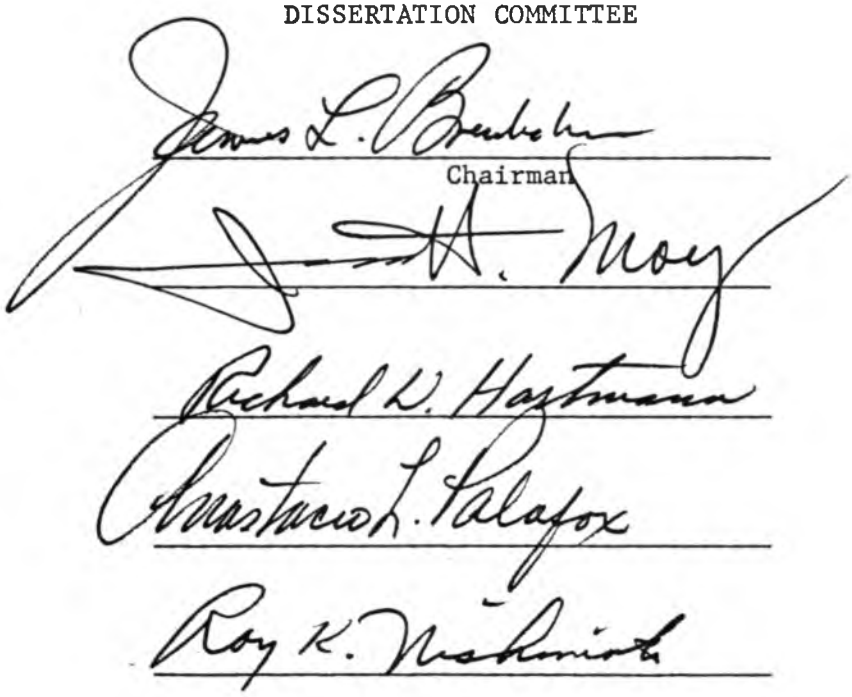

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CHAPTER ONE

SELECTION FOR HIGH AND LOW FIELD VIABILITY

INTRODUCTION

The brittle-2, brittle-1, and shrunken-2 mutants were discovered by Sprague (Emerson et al., 1935), Mangelsdorf (1926) and Mains (1949), respectively. Many years elapsed between their discovery and the recognition by Laughnan (1953) and Cameron (1954) of their high-sucrose property.

The three mutants are extremely sweet by virtue of high levels of sucrose and reducing sugars. An extraordinary feature of these 3 genotypes is that they retain high sweetness and tenderness into late harvest stages. Protein quantities are high, and the protein quality is superior to that of sweet corn. With so many desirable features, including excellent nutritional values, it is impressive that they have been neglected so long. One of the primary reasons is that these mutants have very low germination.

Helm and Zuber (1973) demonstrated that selection could improve the viability of Missouri shrunken-2 stocks. An intensive program for the improvement of many horticultural features of all 3 mutants has been conducted by Brewbaker (1971). Recently Brewbaker released a brittle-2 composite, Hawaiian Supersweet #6, a variety that has shown exceptional horticultural qualities in the tropics. Low field viability remains a major disadvantage of these high-sucrose varieties.

This chapter, therefore, focussed on the genetic improvement in

field viability of shrunken-2 and brittle-2 Hawaiian composite populations.

LITERATURE REVIEW

RECURRENT SELECTION IN CORN

Mass selection for the improvement of corn probably dates back to the time, perhaps 50 centuries ago, when this crop was demosticated. Selection within open-pollinated varieties was a common farmer practice prior to the development of hybrid corn. A widely-used modification of mass selection called ear to row breeding was initiated by Hopkins (1899).

East and Jones (1920) and Hayes and Garber (1919) were among the first to suggest intercrossing selected individuals as a method of concentrating favorable genes in a polygenic system. The first detailed description of this type of breeding scheme was published by Jenkins (1940) as a result of his experiments with early testing for general combining ability in maize. It was not until 1945, when Hull (1945) suggested that selection after each of several cycles of intercrossing might be useful in improving specific combining ability, that the method acquired the name "recurrent selection". Recurrent selection methods can involve either mass (bulk) or pedigree methods of many types but, traditionally, some form of self or sib-pollination with ear to row or pedigree evaluation of progenies is implicit, especially with yield as a major objective.

Recurrent selection involves the continued selection, generation after generation, following interbreeding of selected plants or lines, to provide for maximal genetic recombination (Hull, 1952). Thus selection among individuals or lines is not recurrent until the

selected lines are interbred and a new cycle of selection is initiated. The advantage of this system is that the ceiling performance is set, not by the genotype of a single foundation plant, but by the most favorable combination of genes in a group of foundation plants. The chance of obtaining satisfactory individuals should therefore be increased, compared to selection within selfed or mildly inbred lines, because greater opportunity for recombination is present. Since the rate of inbreeding can, with care, be kept at a low level, it should be possible to maintain high genetic variability and hence provide for effective recurrent selection over a long period.

The first convincing experimental evidence for the effectiveness in changing gene frequencies of two cycles of simple recurrent selection, contrasted with selection within inbred lines, was presented by Sprague and Brimhall (1950). Studying the oil content of corn kernels, they found recurrent selection, on the basis of high combining ability, to be at least 2.5 times as efficient as selection during inbreeding in developing high-oil strains. One cycle of selection for combining ability resulted in a positive shift of 7 bushels per acre in mean yield when related to that of the tester parent.

Lonnquist (1949) obtained highly significant differences between high-yielding and low-yielding synthetics developed from the Krug variety of corn after only one cycle of selection. Plants from these two synthetics were tested in crosses on the single cross WF9 x M14 (Lonnquist, 1951). Frequency distributions of yields for the two populations of test crosses differed greatly, those for the high-yield synthetics having the highest mean and mode.

Frey et al. (1949) reported results of intercrosses for protein improvement among F_3 progenies of the cross Hy x I198. One set of F_3 progenies had been selected on the basis of the ratio of zein to total protein, and a second set had been selected for tryptophane content of the grain. No improvement was realized in the population selected for zein:protein ratio. This was attributed to the lack of sufficient genetic variability, since only three of ten progenies used for intercrossing had a significantly low ratio of zein to total protein. A significant increase in protein was obtained in the population selected for mean tryptophane percentage.

The method of recurrent selection can be modified in many ways to suit special needs. Jenkins et al. (1954), for example, applied simple mass recurrent selection to concentrate genes for resistance to corn leaf blight, Helminthosporium turcicum. Three cycles of recurrent selection were practiced within each of nine progeny groups. Selection proved to be effective in significantly improving the mean leaf blight scores of the first cycle population compared to the original population and also the scores of the second cycle population. The third cycle of selection was less effective than the first two cycles.

Recurrent selection for general combining ability (GCA) variety Krug has been reported by McGill and Lonnquist (1955). The yields of three second-cycle synthetics, derived on the basis of recurrent selection for high GCA, were not significantly different, but each was superior to the parent variety, Krug. Selection for low combining ability appeared to be somewhat less effective. Results from four cycles of recurrent selection for general combining ability for yield

in corn were reported by Penny et al. (1963). Their results suggested an increasing yield trend with an average of 1.2 percent per cycle.

Johnson (1952) reported results obtained from the first cycle of recurrent selection for combining ability for yield in sweet clover, Melilotus officinalis. The results suggested that the procedure offers considerable promise in forage crop improvement.

A breeding procedure designated as reciprocal recurrent selection was proposed by Comstock et al. (1949). The proposed scheme was designed to make maximum use of both general and specific combining ability, regardless of the type of gene action involved, in improving both parents of a cross simultaneously.

A more recent report by Twamley (1974) indicated that progress in seedling vigor improvement of about 35 to 40% in 'Leo' birdsfoot trefoil (Lotus corniculotus L.) was made by three cycles of full-sib pedigree recurrent selection.

Mass selection for seedling survival in a Missouri shrunken-2 (sh_2) population (Mo.sh_2) was investigated by Helm and Zuber (1973). The Mo.sh_2 population underwent 8 cycles of pedigree ear to row selection for seedling survival. Additional selection pressure was applied in the last 4 cycles for kernel weight. Germination and seedling survival was tested for 2 planting dates at 4 corn belt locations in 1971. Survival was still less than for dent corn. The sh_2 population was similar in germination and survival to commercial sh_2 hybrids marketed in the corn belt and both were significantly better than a sh_2 hybrid of conventional corn belt material. After 8 cycles of selection, sh_2 had a seedling survival of 55% as compared to

N15$\underline{sh}_2$ x B37$\underline{sh}_2$ F_1 which had 19% survival. Kernel weights were correspondingly increased during selection, from .08g to .16g/kernel vs. 0.60g for the dent hybrid.

Lindstrom (1943) reported that inbred lines of dent corn showed remarkable differences in germination when stored for 12 years under laboratory conditions. He concluded that seed longevity in inbred lines of dent corn has a hereditary basis, but inheritance did not appear to be simple. The capacity of seed of various inbred lines of sweet corn and their hybrids to retain ability to germinate, as reported by Haber (1955), varies greatly under common storage conditions. Humidity and temperature are the major environmental factors which determine longevity, but there is also a genetic basis for longevity, as supported by the statements of Lindstrom (1943). Haber found that in general the longevity of the hybrids was better than the same two inbreds which were used to make up a single cross hybrid. Inbred IP39 maintained longevity of seeds reasonably well as did inbred Iowa 45. Garner (1921) reported germination of sweet corn seeds harvested prematurely (at milky stage when moisture content is between 55 and 60%), was severely reduced.

Most of the reports in the literature clearly indicate that progress from intra-population selection can be expected if the source materials have adequate genetic variability. Appropriate field plot techniques are required so that genotypic differences are reflected in the phenotypic variation on which selection is based. On the assumption that field plot techniques were a limiting factor in rendering the ear to row method useful, some modifications were

suggested by Lonnquist (1964). The most obvious limitation of mass selection as a method of population improvement is that it is based upon phenotypic selection of the plant in a single location or planting. The ear to row procedure offers a means of increasing accuracy in intra-population selection. The modified procedure by Lonnquist is essentially between and within family selection programs. The family choice is dependent upon the average performance in 3 locations. The choice of individuals, within selected families, is based upon visual selection at one location.

It must not be overlooked that with respect to field corn, a breeder's main fixation is on yield, whereas high-sucrose breeding is much less preoccupied with yield.

MATERIALS AND METHODS

Materials used to determine the effect of planting date on field viability of corn are presented in column 1 of Table 1. The H609 and H68 are single crosses (SX), H676 is a double cross (DX), HS is a variety and the rest of the entries are composites (COMP) and synthetics (Syn).

For the selection experiments, parental materials used were $\underline{sh}_2$ composite 2g and $\underline{bt}_2$ composite 1e. From approximately 200 crosses, 120 ears were selected on the basis of good tip cover, ear size and shape, kernel size, freedom from ear rot and worm damage. Each of the selected ears was further inspected to remove seeds that were diseased, mechanically damaged or immature. The ears were then individually shelled and duplicate samples of 100 seeds were weighed into manila envelopes. Seeds were counted with the aid of an electric seed counter and a hand-crafted board with 100 holes on the surface (Figure 1b).

Measurements were made of seed volume. From these data, density was calculated for duplicate samples of 50 seeds each, by dividing the weight of the sample by its volume. Volume was obtained by a special apparatus designed to measure water displacement (Figure 1c). The apparatus consisted of an upright plastic bottle, 15.5 cm high and 6.5 cm in diameter, with upper portion cut. The bottle was placed at the edge of the desk or table. The cylinder had a drain spout located 5 cm below the top edge. A small plastic tube 3 cm long emerged from the spout at a 90° angle. The sample holder was a light, perforated, aluminum tea bag container, acorn-shaped and 5 cm in diameter (Figure 1).

Figure 1. Apparatuses for density measurements and seed counting.

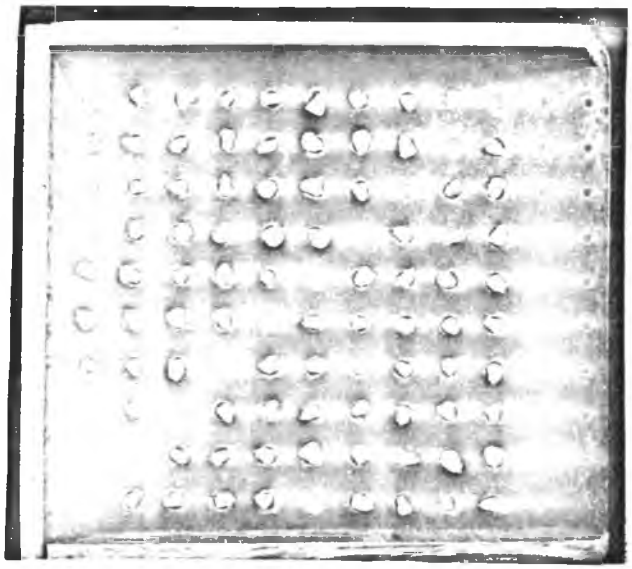
(a) Sample container

(b) Seed counter

(c) Apparatus for measuring density

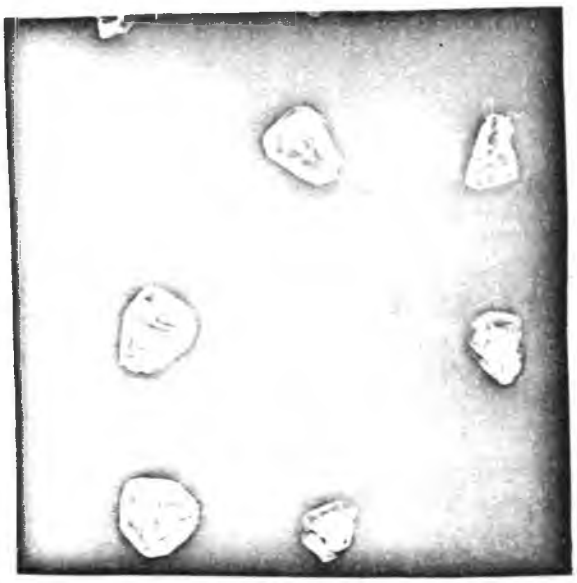
(d) Seed counter (close up)

(a)



(b)

(c)



(d)

A chain was attached to the lid, allowing the samples to be lowered into the cylinder. The cylinder was filled with water, and the excess water was allowed to drain through the plastic tube. A 50 kernel sample of $\underline{sh}_2$ corn was then placed into the tea holder. The sample was then lowered into the cylinder and the displaced water overflowed through the protruding tube into a 25 ml graduated cylinder. Volume displacement was recorded after subtracting the volume of the tea holder. The corn samples used in the test were previously dried for 10 days in order to bring them to a constant weight. Density measurements were taken only in the second cycle of selection.

Cycle-0 consisted of seeds from the full-sibbed ears; these were planted ear to row in 2 replications, one month apart at the Waimanalo Experimental Station. Each replication was made up of 120 short plots (20 feet long), each plot containing 100 kernels. The first replication was planted on April 9, 1973, in field S1 and field counts were made 26 days after planting. Short rows with seedling emergence between 27% and 60% were rogued out. From this initial population, selections were made in two directions, high (above 60%) and low (below 27%).

Thinning of seedlings within chosen lines was carried out over a 6 week period. This allowed for extensive selection against early and late mosaic, poorly rooted plants (no brace roots), poor pollen procedures, multiple ear plants and plants with high ear placement. In each cycle, vigorous seedlings from the highest or lowest germinating lines were selected to be the parents of the next cycle.

The second replication was planted on March 9, 1973, in the same field. Identical field practices were applied for this planting.

RESULTS AND DISCUSSION

1. Effect of planting date on field viability of corn

Variation in field emergence of 14 lots of field, sweet and supersweet corn was observed in three consecutive winter plantings (Table 1). All entries were planted at the Waimanalo Farm in 2 replications each on January 13, February 13 and March 13, 1973. Duplicate samples of each of the fourteen lots were germinated in an incubator at a temperature of 86°F, to determine germination percent.

Germination avraged 61% in January, 74% in February and 68% in March. Average minimum and maximum ambient temperatures during the 3 months were 65 and 78, 64 and 77, 68 and 78°F. Despite the fact that the temperatures for the 3 winter months remained approximately the same, the mean germination percent of the 14 entries was low in January and increased in February. Unlike January and February, discrepancies between the two reps were observed in March, which presumably resulted from large variations in germination between the entries.

Average germinations were 70% and 75% for field and incubator, ranging from 41% to 87% in the field, and from 53% to 97% in the incubator. All high-sucrose mutants were relatively low in germination, in the field as well as in the incubator. However, viability was observed as high as 72% in some instances ($\underline{fl_2}\underline{sh_2}$ COMP 1b). Field germination rates averaged 93% of those in the incubator, but were generally lower for the high-sucrose lines (Table 1).

Average kernel weight of the 14 entries was 15.67 grams. Kernel weights of all high-sucrose varieties were lower (12 gm./100 kernels)

Table 1. Germination percentages of 14 maize genetic stocks in 3 winter months of 1973.

Entries	Germination Percentages on Planting Dates						Overall Avg.	Wt. of 100 kernel in gm.	Incub. Germ	Ratio F/I
	Jan. 13, '73 Reps	Avg.	Feb. 13, '73 Reps	Avg.	Mar. 13, '73 Reps	Avg.				
H609 (+)	80,85	82	99,90	94	82,69	75	83 ab	29.80	90	0.92
H676 (+)	80,63	72	85,83	84	89,85	87	80 ab	26.2	80	1.00
H68 (su	88,80	84	97,97	97	71,92	81	87 a	17.1	97	0.90
HS (su)	72,82	77	95,83	89	92,85	88	84 ab	19.1	87	0.96
$o_2$su COMP 1e	92,99	95	81,97	89	78,71	75	86 a	17.5	90	0.96
bf_1 COMP 1e	37,37	37	52,61	56	70,51	60	51 ef	11.5	62	0.82
bt_1 COMP 2e	43,34	38	51,54	52	38,31	34	41 f	10.7	53	0.77
bt_2 COMP 1d	37,32	34	71,47	59	73,30	51	48 f	13.1	62	0.77
sh_2 Syn 1f	41,53	47	63,74	68	65,52	58	58 de	12.3	70	0.83
sh_2 Syn 2g	57,42	50	68,80	74	79,65	72	65 cd	11.3	75	0.86
sh_2 COMP 1g	40,42	41	63,43	53	68,52	60	51 ef	12.4	65	0.78
sh_2 COMP 2f	62,68	65	56,66	61	54,57	55	60 de	15.5	67	0.90
sh_2 COMP 3d	71,63	67	70,80	75	69,76	72	71 bc	10.0	75	0.95
fl_2sh_2 COMP 1b	60,80	70	70,87	78	80,60	70	72 bc	12.9	79	0.91
Average	61,61	61	73,74	74	72,65	68	70	15.67	75	0.93
DLSD @ 0.05				7.79						
r							-0.12		0.03	
Avg. Min. Temp.		65°F		64°F		68°F				
Max. Temp.		78°F		77°F		78°F				

than either field (27 gm./100 kernels) or sweet corn (17 gm./100 kernels). There was no correlation of seed weight and germination percent of high-sucrose corn in the field r=-0.12 or in the incubator r=0.03.

Variance analysis (Table 2) revealed the significance of entries main effect and entries x date interaction. Bayes LSD indicated that all of the sweet and field corn were significantly better in germination than supersweet corn, except for $\underline{fl_2 sh_2}$ COMP 1b and $\underline{sh_2}$ COMP 3d. Several other factors, moisture, soil aeration and soil micro-organisms influence germination percent, but their role here appears to have been negligible, and of similar magnitude in all three groups of corn.

This preliminary experiment clearly gave evidence that viability of field and sweet corns is superior to that of supersweet varieties. This suggested the possibility of selection experiments designed to improve the germination of supersweet varieties.

2. Selection for high field viability in a Hawaiian shrunken-2 ($\underline{sh_2}$ population through 2 different recurrent selection schemes

Parental material used for the two schemes of selection was $\underline{sh_2}$ COMP 2g (Brewbaker, 1971). Cycle 0 for this composite was made in 1973 by sib-pollinating (full-sib matings) the most vigorous plants. The 120 ears constituting the original population were planted in April 1973. Plantings for cycle 1, cycle 2 and cycle 3 were made in August 1973, December 1973 and May 1974. Within the 24 highest germinating lines in cycle 1 both full-sib matings (scheme I) and selfing (scheme II) were practiced to produce 2 populations of 120 lines each. In scheme I both cycle 2 and cycle 3 selected lines were sibbed. In scheme II all lines

Table 2. Analysis of variance for average germination percents in Table 1.

Source	df	SS	MS	F
Experiments (D)	5	2,102	420	
Entries (E)	13	19,492	1,499	25.6**
E x D	26	4,561	175	2.9**
Error	39	2,284	59	
Total	83	28,439		

were sibbed in cycle 2 and self-pollinated in cycle 3. The selection indices for schemes I and II were 33% and 33% in cycle 1 and 45% and 36% in cycle 2 respectively.

Frequency distributions of ears are presented in Figure 2. Population means are indicated by solid vertical lines and means of the ears chosen as parents of the next cycle by broken vertical lines.

In the results from the first cycle, the mean of this population exceeds slightly the mean of the selected parents. This would not be expected and undoubtedly results from the vagaries of sampling.

In the second cycle, the mean was further shifted to the right by an amount equal to 13.9%. In spite of this change in gene frequency, the mean of this sample did not equal the mean of the selected parents within the first cycle.

In the third cycle the mean was slightly shifted to the right by an amount equal to 2.5%.

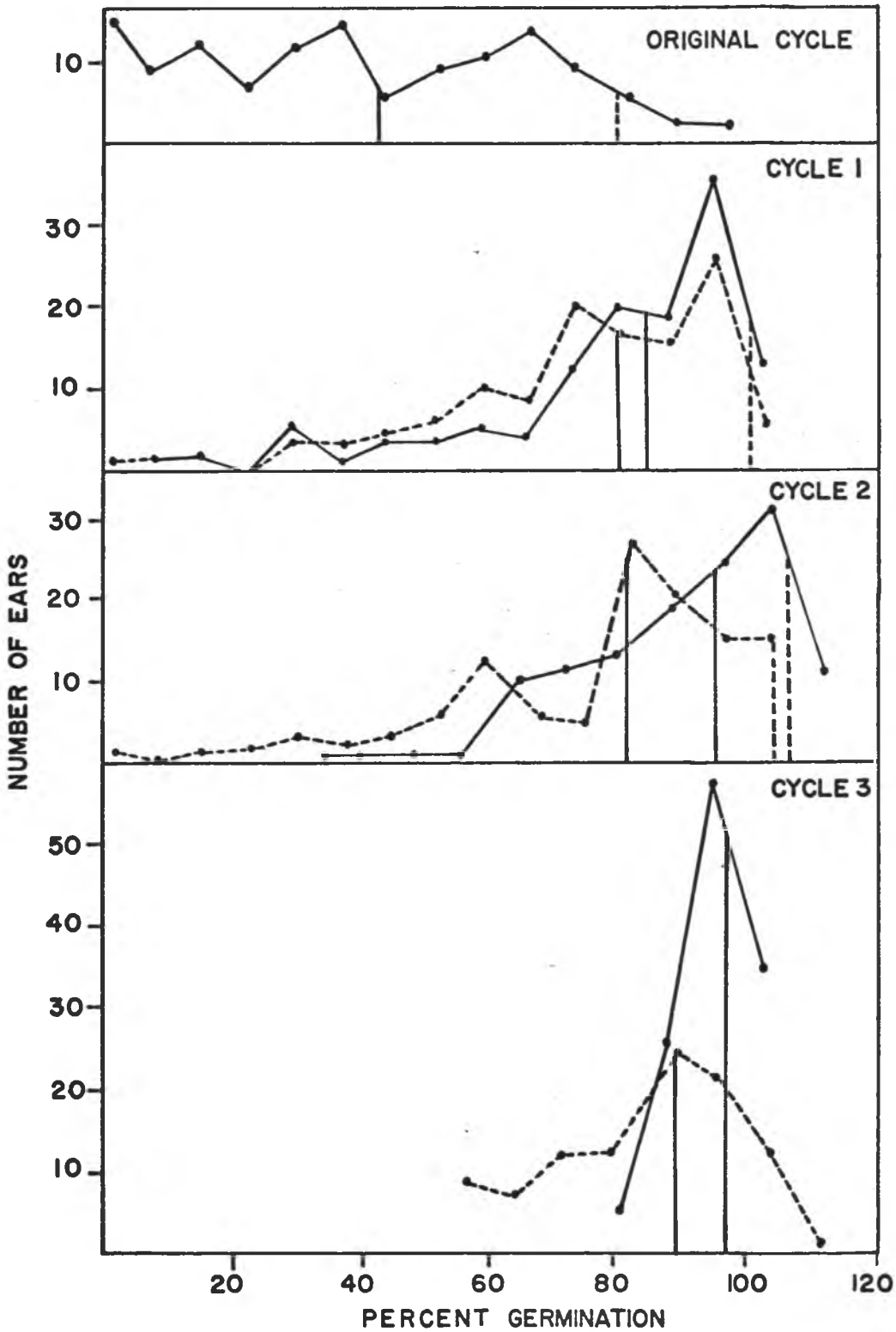
The mean of the original population (as percent of H68) was 43.8%. The mean of the first cycle population was 83.6%. The means of the second and third population cycles (scheme I) were 95.3% and 97.7% respectively.

The ranges in the four populations are of some interest in indicating any change in genetic variability. In the original population, the range was from 0% to 92%, in the first cycle 10% to 97%, in the second cycle 32% to 100% and in the third cycle 78% to 100% or ranges of 92, 87, 72 and 22%, respectively. The original population had the greatest standard deviation (24.5), the first cycle was intermediate (18.5), the second (6.7) and the third cycle (5.1) had

Figure 2. Frequency distributions of germinations (in percentage of H68 check) of $\underline{sh}_2$ COMP 2 populations following 2 schemes of selection.

_____ Scheme I

- - - - Scheme II


ORIGINAL CYCLE
CYCLE 1
CYCLE 2
CYCLE 3
NUMBER OF EARS
PERCENT GERMINATION
10
30
20
10
30
20
10
50
40
30
20
10
20
40
60
80
100
120

the smallest.

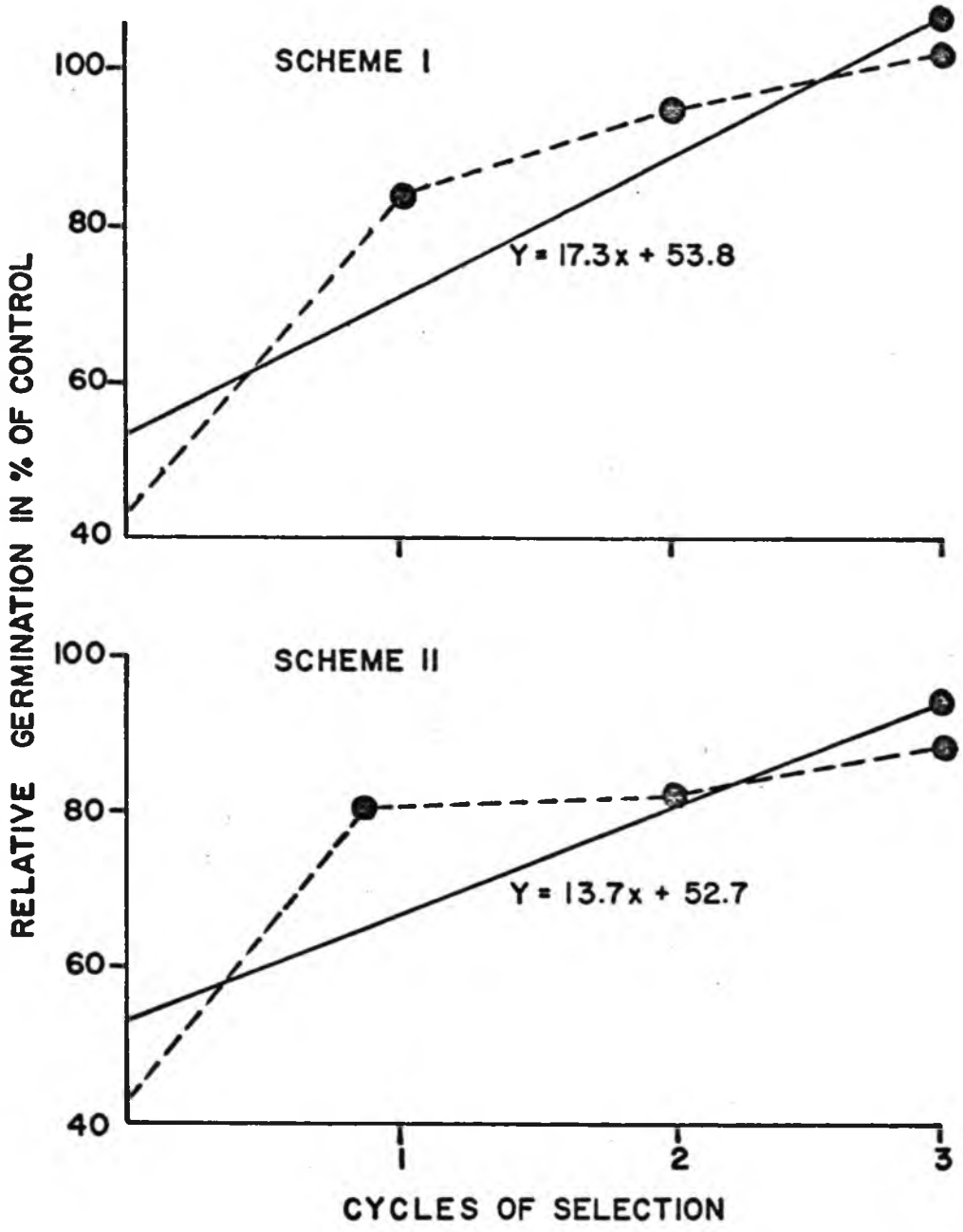
An estimate of average progress per cycle is provided by the linear regression coefficient, b=13.7% (Figure 3). The full advantage of the selected parents for kernel weights in scheme I was retained in scheme II in the first and second cycle population and decreased in cycle 3. The reason for the lack of progress in seed weight for cycle 3 was due to adverse winter weather conditions in which cycle 2 progenies were produced. During the entire period of growth and development, this cycle was subject to continuous winter rain-fall and low light intensity. Both the development of ears and kernels were reduced. In spite of the fact that seed weight decreased in cycle 3, germination rates still increased. This indicates that germination is independent of seed weight and is genetically controlled.

The hybrid H68 was used as a check in all selection experiments. Data revealed that a difference of 57% existed between the original population, $\underline{sh}_2$ COMP 2g and H68. This difference was gradually reduced to 16%, 5% and 3% over the remaining 3 cycles of selection.

The germination data for each of the cycles of scheme I, relative to the hybrid H68, are presented graphically in Figure 3. The linear regression of germination percent, on cycles of selection, calculated from the available data, was 17.3% and was highly significant.

The results obtained with scheme II of recurrent selection for cycles 1, 2 and 3 are presented in Figure 2 (broken lines). The frequency distributions of the first, second and third cycles ran parallel to those of scheme I, however large variations did exist in the third cycle of scheme II. The mean germination percentage shifted

Figure 3. Average relative germinations after each of 3 cycles of ear to row selection in $\underline{sh}_2$ COMP 2g population together with linear regression fitted to the germination mean of scheme I and II.


SCHEME I
Y = 17.3x + 53.8
SCHEME II
Y = 13.7x + 52.7
100
80
60
40
1
2
3
RELATIVE GERMINATION IN % OF CONTROL
CYCLES OF SELECTION

rapidly upwards; from 43.8% in the original cycle to 80.1%, 82.4% and 88.5% in cycles 1, 2 and 3 respectively.

The range in germination varied to a large extent in the original 3 cycles, but narrowed considerably in the final cycle of selection. The standard deviation was 19.7 for cycle 1, 9.7 for cycle 2 and 12.0 for cycle 3. The data revealed that a difference of 57% existed between the original population, $\underline{sh}_2$ COMP 2g and H68. This difference gradually reduced to 20%, 18% and 12% over the remaining 3 cycles.

Neither kernel weight nor seed density were significantly correlated with germination percentage in cycle C-2; r=0.09 germination (y) vs weight (x) and r=0.050 germination (y) vs density (x) for scheme I and r=0.46 and r=0.000 for scheme II respectively. These correlations represent only the calculations for cycle 2. The correlation coefficients of germination percentage and kernel weight for the 4 populations are presented in Tables 3a and 3b.

At the termination of the selection experiments in May 1974, random seed samples from each cycle of the two schemes were bulked to provide 7 treatments including H68 as a check (Table 4). All treatments were germinated in the incubator (3 samples of 100 kernels), in flats (5 samples of 50 kernels) and in the field (5 samples of 200 kernels).

Bulked seeds from each cycle varied greatly in germination, although seeds placed under the more controlled environmental conditions, incubator and flat, germinated better than those in the field. Germination tests in the incubator were observed to be no better than in the flats (Table 4). One possible reason is the $\underline{sh}_2$ kernels have high concentrations of sugars, and seeds which are very slow to germinate

Table 3a. Statistics of variation calculated for kernel weight and germination percentages in the selection experiments.

$\underline{sh}_2$ COMP 2g scheme I

	Planting Date								
	April 9 C 0		Aug. 27 C 1		Dec. 18 C 2*			May 6 C 3	
Statistics	wt.	germ.	wt.	germ.	wt.	germ.	dens.	wt.	germ.
n	120		120		120			120	
$\bar{x}$	11.8 gm	39.4%	14.4 gm	76.9%	14.9 gm	79.1%	1.0 gm	11.4 gm	89.9%
range	6.6-17.5	0-92%	9.9-20.7	10-97%	11.2-19.0	32-100%	0.8-1.3	6.5-16.8	78-100%
s	2.6	24.5	1.8	18.5	0.8	6.7	0.1	2.1	5.1
$s_{\bar{x}}$	0.2	2.2	0.2	1.7	0.1	6.6	0.0	0.2	0.5
CV	22.0%	62.2%	12.5%	24.0%	5.4%	16.9%	10.0%	18.4%	5.6%
r	0.60		0.25		0.09		0.050	0.10	
in % of H68		43.8%		83.6%		95.3%			97.7%
H68		90%		92%		83%			92%

*50 seeds

Table 3b. Statistics of variation calculated for kernel weight and germination percentages in the selection experiments.

$\underline{sh}_2$ COMP 2g scheme II

	April 9 C 0		Aug. 27 C 1		Dec. 18 C 2*			May 6 C 3	
Statistics	wt.	germ.	wt.	germ.	wt.	germ.	dens.	wt.	germ.
n	120		120		120			120	
$\bar{x}$	11.8 gm	39.4%	13.3 gm	72.1%	13.6 gm	73.3%	1.0	10.0 gm	74.3%
range	6.6-17.5	0-92%	9.8-18.5	3-98%	9.9-17.8	6-100%	0.8-1.5	5.1-14.4	49-95%
s	2.6	24.5	1.8	19.7	0.9	9.7	0.1	2.1	12.0
$s_{\bar{x}}$	0.2	2.2	0.2	1.8	0.1	0.9	0	0.2	1.1
CV	22.0%	62.2%	13.5%	27.3%	6.6%	13.2%	10.0%	21.0%	16.2%
r	0.60		0.19		0.46			0.19	
in % of H68		43.8%		80.1%		82.4%			88.5%
H68		90%		90%		89%			84%

*50 seeds

Table 4. Average germination rates of bulked samples of ear to row progenies and of the control.

	Germination rate in percent					
	Incubator		Flat		Field	
Cycles	I	II	I	II	I	II
1	75 ef	68 f	58 h	74 ef	67 fg	62 gh
2	85 ab	75 ef	79 bcd	76 ef	81 bcd	77 cd
3	86 ab	90 a	90 a	92 a	78 bcd	76 ef
Loc Avg.	80		78		73	
H68 Control	88		96		91	
DLSD @ 0.01	8.02					

attract rotting and putrifying organisms. Analysis of variance (Table 5) confirmed the significance of differences of the main effects (except for schemes) and first and second order interactions (except for scheme x cycle).

Lack of significance between schemes I and II indicates that both schemes were about equally effective in changing the gene frequency. Progress in germination improvement of 54% was made in three cycles of recurrent selection in scheme I and 46% in scheme II. The slightly slower progress made by scheme II may be attributed to two factors; selfing eliminated the homozygous albino-lethals and these could not have been observed in the incubator where germination counts were made on the basis of a half-inch emergence of the radical; secondly was the presence of the $\underline{su}_1$ gene, which upon selfing, segregated 3 $\underline{sh}_2+$ and 1 $\underline{sh}_2\underline{su}_1$, the latter being extremely poor in germination.

As a result of this data collection, it was determined that germination in the incubator and in the flat is significantly better than germination in the field for the 3rd cycle. The reason for the decrease in germination in the field was due to the poor quality of seed and to the fact that undesirable traits are not expressed under the more controlled conditions of the incubator and flat (Figure 4).

Several factors may be listed as important in determining the efficiency of selection; these are heritability, sample size, gene frequency, maternal effect and dominance of deleterious genes. In each of the first cycle populations, heritability for germination percentage was high. Sample size seems to have been adequate for the purpose of high viability selection.

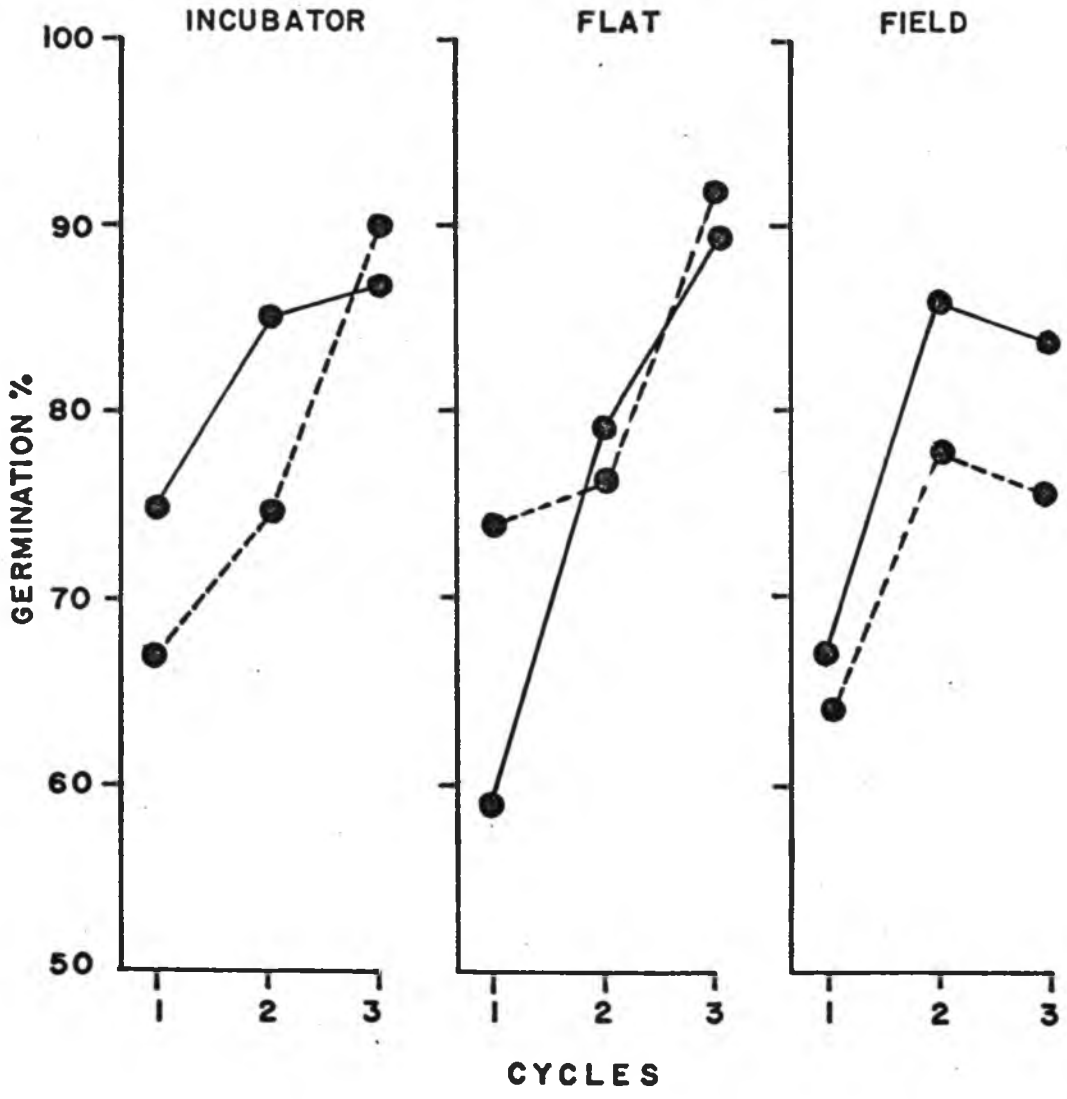
Table 5. Analysis of variance from Table 4.

Source	df	SS	MS	F
Schemes (S)	1	6.2	6.2	0.2
Cycle (C)	2	4,586.8	2,293.4	83.1**
Location (L)	2	565.8	282.8	10.2**
S x C	2	213.8	106.9	3.8
S x L	2	387.7	193.8	7.0**
C x L	4	2,374.0	593.5	21.5**
S x C x L	4	710.5	177.6	6.4
error	60	1,653.8	27.6	

Figure 4. Average germination percentages of bulked seeds from 3 cycles of selection by means of two selection schemes germinated in 3 locations.

———— Scheme I

-------- Scheme II


INCUBATOR
FLAT
FIELD
100
90
80
70
60
50
GERMINATION %
1
2
3
CYCLES

Gene frequency is known to have an effect on the efficiency of selection. Selection is less effective when gene frequencies (q) are near zero or 1 and most effective when q is closer to 0.5. If the gene frequencies are above 0.5 then any increase will lead to a decrease in variability. This may be a possible explanation for the decreased variability observed in each of the first cycle populations.

The progenies were full-sibbed. Despite the fact that the viability of the male parent seeds were not known, it must be argued that there is no maternal effect. The fact that selfing gave no advantage argues against maternal effect.

The first generation of recurrent selection was extremely effective in improving the mean germination percentage of the resulting sub-populations. The effects of the last two cycles of selection were more or less negligible. The use of modified ear to row procedure with pedigree selection from sibbed or selfed ears proved to be effective in the selection of genotypically superior individuals. The method is essentially a combination of progeny testing, with selection based on family means. As pointed out by Lerner (1958) the gains expected from combined methods of selection should be higher than those obtained from either mass selection or family selection alone.

These results are similar to those of Sprague and his colleagues (1952), Jenkins et al. (1954) and Twamley (1974) who showed the effectiveness of recurrent selection in increasing the "superior" genes in a gene pool. The $\underline{sh}_2$ viability data are distinguished by the major reduction of genetic variability caused by one cycle of selection for high germinating lines. Selection for oil content was accompanied by

little loss in genetic variability. It is possible that so few genes govern reduced germination in $\underline{sh}_2$ COMP 2 that their frequency was decreased to a point where little genetic variability remained after one or two cycles of recurrent selection.

It is possible that semi-lethal genes played an important role in the viability of the $\underline{sh}_2$ population. Lethal genes are exposed in the presence of the $\underline{sh}_2$ gene. A perfect example for the exposure of lethal genes is the sugary-1 gene in a $\underline{sh}_2$ homozygous condition. Sugary-1 expresses no lethal effect on viability, but in the presence of homozygous $\underline{sh}_2$, germination is drastically reduced. Either selfing or sibbing during the first cycle of selection might have exposed a large frequency of semi-lethal genes due to the fact that the $\underline{sh}_2$ COMP 2 population carried a high frequency (est. <30%) of the sugary-1 gene. This may account for the rapid decrease in the genetic load.

The improvement by selection in germination suggests rapid single generation advance by ear to row method, either by sibbing or selfing in the field or flat. It is recommended that this be a regular feature of all $\underline{sh}_2$ or $\underline{bt}_2$ selection programs--i.e., one generation out of every 3rd or 4th, plant ear to row carefully dropping 4 seeds/hill in 25 hills. Eliminate all lines under 70% germination. Selection intensity of 25% or less is recommended.

Further research may be needed in order to determine whether improvement in germination is accompanied by changes in quality of supersweet corn (tenderness, flavor, sweetness and amount of protein), but there was no intimation of such changes in this study.

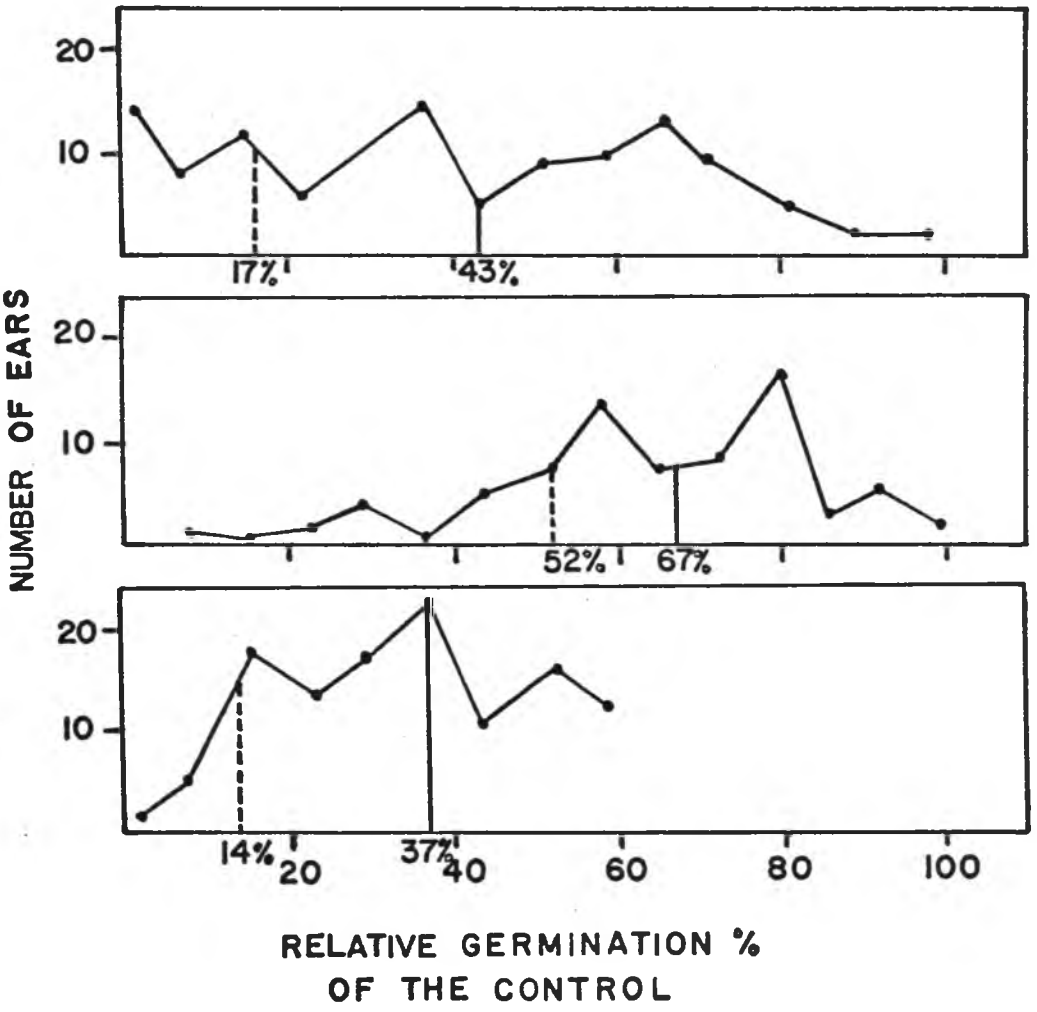
3. Selection for low viability in Hawaiian shrunken-2 (sh_2) population through full half-sib pedigree selection

Shrunken-2 COMP 2g (Brewbaker, 1971) was also used for the selection of low viability. From 120 lines of cycle 0, 20 foundation lines with germination percent 26% and below were sib-pollinated (full-sib matings with pedigree selection). Seventy-nine ears were recovered and planted ear to row on August 27, 1973 in field A1. Low germinating rows were sib-pollinated 1 on 1+ to produce 120 ears. The second cycle was planted on May 4, 1974. Selection intensities for cycle 0 and 1 were 17% and 28% respectively. The frequency distributions for the original population (120 ears), cycle 1 (79 ears) and cycle 2 (120 ears) were derived from grouping into 15 classes of 6.67 per class interval (Figure 5). The solid vertical lines represent the sample mean and the dotted lines, the mean of the selected parents.

Results from the first cycle of selection for low viability show that the mean of the population greatly exceeded the mean of the selected parents. This upward shift, rather than downward, in germination percent was unexpected and presumably results from sampling error. In the second cycle the mean of the population shifted to the left. Mean germinations in percent of the control (H68) were 43.8%, 67.3% and 36.8% for cycle 0, cycle 1 and cycle 2 respectively.

In the original population, germination ranged from 0% to 93%, 10% to 98% in the first cycle and 2% to 29% in the second cycle. It is of interest to note that in each of the three populations, variability in the original cycle was greater than that of the first and second population as measured by their standard deviation (s=24.5, 19.6 and

Figure 5. A comparison of frequency distributions of germination percentage in the original population, and after 2 cycles of recurrent selection.


NUMBER OF EARS
20
10
17%
43%
20
10
52%
67%
20
10
14%
37%
20
40
60
80
100
RELATIVE GERMINATION %
OF THE CONTROL

14.2 for cycle 0, 1 and 2 respectively). Selection response for low germination percent is diagrammed in terms of the linear regression line (Figure 6). The linear regression coefficient for germination, calculated for the 2 cycles of selection was -3.0%, and was not significant.

No significant correlation between low germination percent and kernel weight or density was found (Table 6). An increase in seed weight occurred from cycle 0 to cycle 1; however, mean seed weight remained constant from cycle 1 to cycle 2. Increase in seed weight from selected low germinating ears, may be attributed to the fact that the parental stocks were grown under ideal environmental conditions. No observable differences were detected between seeds or seedlings from high or low germinating lines. Due to the fact that the plants in the low germinating lines did not suffer the consequence of crowding, as in the high lines, plants had better stature and were more vigorous.

4. Selection for high field viability in Hawaiian brittle-2 (bt_2) population

The material used in this experiment was brittle-2 (bt_2) COMP 1 (Brewbaker, 1971). The initial population consisted of approximately 4,000 plants. Cycles 0, 1 and 2 were planted on June 7, 1973, November 10, 1973 and March 29, 1974 respectively. The selection index for cycle 0 was 25% and for cycle 1, 33%. Mass selection was practiced on the original population whereas a sib 1 on 1+ procedure was applied to the first and second cycles.

Results obtained from full-sib pedigree selection are presented in

Figure 6. Average relative germination after each of 2 cycles of ear to row selection in $\underline{sh}_2$ COMP 2g population together with linear regression fitted to germination mean.

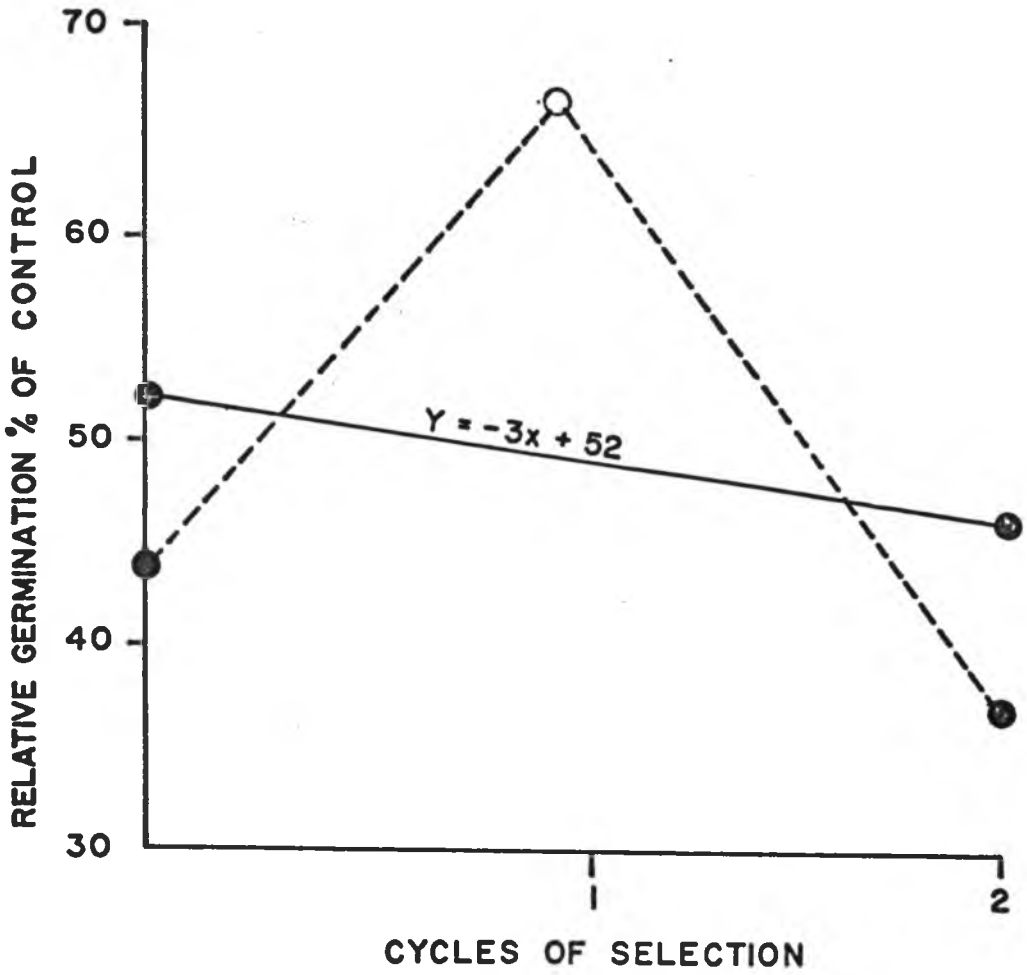

70
60
50
40
30
RELATIVE GERMINATION % OF CONTROL
Y = -3x + 52
1
2
CYCLES OF SELECTION

Table 6. Statistics of variation calculated for kernel weight and germination percentage in the selection experiment for low germination in $\underline{sh}_2$ COMP 2g.

	Planting Date						
	April 9, 1973 C 0		Aug. 27, 1973 C 1		May 4, 1974 C 2		
Statistics	wt.	germ.	wt.	germ.	wt.	germ.	dens.
n	120		79		120		
$\bar{x}$	11.8 gm	39.4%	13.8 gm	61.9%	13.8 gm	33.9%	1.1
range	6.6-17.5	0-92%	8.7-17.8	10-98%	9.6-18.5	2-29%	0.9-1.1
s	2.6	24.5	1.7	19.6	1.8	14.2	0.1
$s_{\bar{x}}$	0.2	2.2	0.2	1.8	0.2	1.3	0
CV	22.0%	62.2%	12.3%	31.7%	13.0%	41.9%	9.0%
r	0.60		0.17		0.28		-0.150
in % of H68		43.8%		67.3%		36.8%	
H68		90%		92%		92%	

Figure 7. In each distribution, the mean is indicated by a solid vertical line and the mean of the chosen parents for the next cycle, by a broken vertical line. The distribution curves are determined on percent basis of the sweet corn hybrid H68.

One of the striking features is the marked shift in the mean from 40% to 70%. The result of the first cycle indicated that the mean of this population equalled the mean of the selected parents. In the second cycle the mean further shifted to 76%, an advance which was much less than that which occurred from the original cycle to the first. Individual progeny germinations ranged from 6% to 93%, 9% to 99% and from 12% to 94% for the 3 cycles respectively. All 3 populations exhibited similar ranges and standard deviations of 19.9, 19.1 and 19.2 for the original population, cycles 1 and 2 respectively.

In order to provide a clearer picture of the progress made by recurrent selection, the data is presented graphically in Figure 8. The best estimate of average progress, per cycle, made to date is provided by the regression coefficient and is 18%.

Mean weights of 100 kernels for the 3 cycles were 15.7 gm., 12.7 gm. and 14.2 gm. respectively. Kernel weight ranged from 11.8 to 19.1 gm. for the original population, 8.7 to 17.8 gm. for the first cycle and 9.6 to 18.5 gm. for the second cycle.

All attempts to correlate germination percentage with kernel weight or kernel density have failed. The correlation coefficients of germination percent (y) vs kernel weight (x) were r=0.16 for the original cycle, r=0.19 for the first cycle and r=-0.08 for the second cycle. Correlation of germination percent vs kernel density, for the second

Figure 7. A comparison of the frequency distributions of germination percent in the corn kernel of $\underline{bt}_2$ COMP 1e for three cycles of recurrent selection.

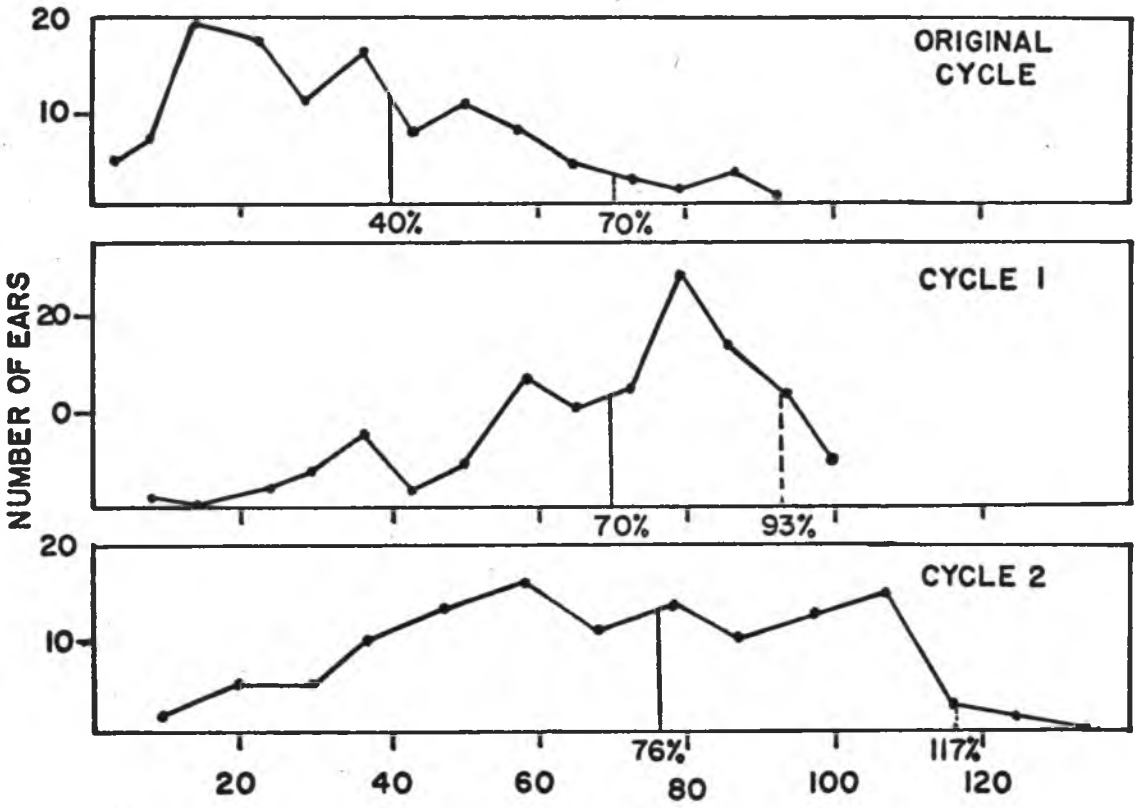
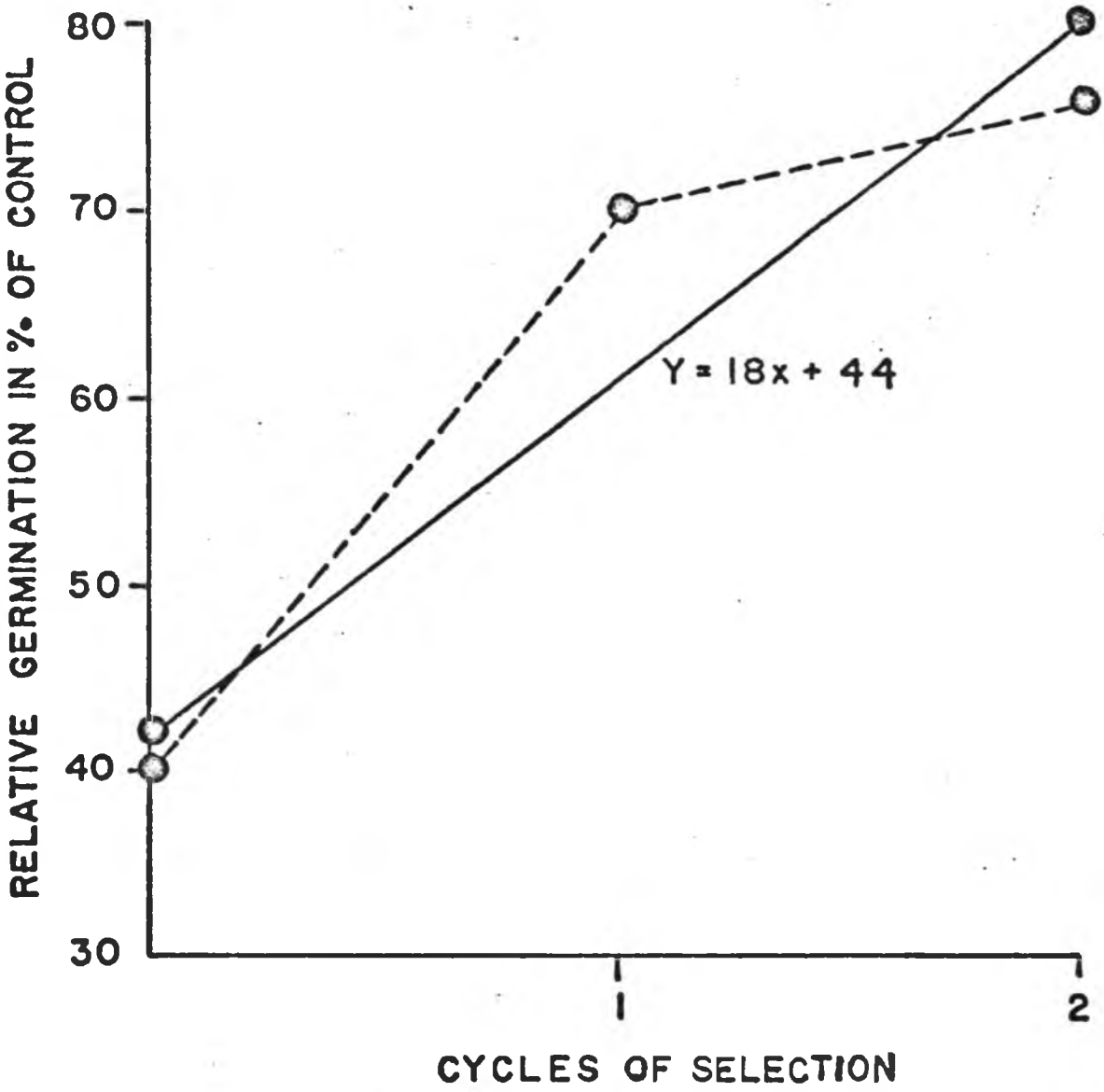

ORIGINAL CYCLE
CYCLE 1
CYCLE 2
NUMBER OF EARS
20
10
0
40%
70%
93%
76%
117%
20
40
60
80
100
120
RELATIVE GERMINATION AS PERCENT OF THE CONTROL

Figure 8. Average relative germinations after each of 2 cycles of ear to row selection in $\underline{bt}_2$ COMP 2e population together with linear regression fitted to the germination mean.


80
70
60
50
40
30
RELATIVE GERMINATION IN % OF CONTROL
Y = 18x + 44
1
2
CYCLES OF SELECTION

cycle only, was r=0.030. Other statistical parameters calculated in relation to kernel weight are shown in Table 7.

As estimated from the population means, substantial progress was made in increasing germination percent of the $\underline{bt}_2$ composite. The gains in field viability over the base population for the two cycles were 75% and 90% respectively. Major progress in germination percent was achieved in the first cycle of selection, with more or less negligible effect in the second cycle. High heritability for germination and the use of a modified ear to row procedure with pedigree selection proved to be effective in the selection of genotypically superior individuals.

These results are similar to those obtained in the $\underline{sh}_2$ COMP 2 populations and show the effectiveness of recurrent selection in increasing desirable viability genes in a gene pool. It is again possible that so few genes control the reduced germination in $\underline{bt}_2$ COMP 1e that their frequency was decreased to a point where little genetic variability remained after one or two cycles of recurrent selection.

Rapid increase in germination during the first cycle of selection, followed by a protracted period of slow progress in the second cycle is probably associated with increases in the frequency of a small number of important genes. The subsequent slow progress of cycle 2 is presumably associated with further small changes in the frequency of the major genes and with slow increases in the frequency of minor genes. It is expected then, under these assumptions, that changes in gene frequency (Δq) will be small, even at intermediate gene frequencies, for single members of systems of genes will each contribute small effects, and hence progress under selection will also be slow.

Table 7. Statistics of variation calculated for kernel weight and germination percentage in the $\underline{bt}_2$ selection experiment.

	Planting Date						
	June 7, 1973		November 10, 1973		March 29, 1974		
	C 0		C 1		C 2		
Statistics	wt.	germ	wt.	germ.	wt.	germ.	dens.
n	120		120		120		
$\bar{x}$	15.7 gm	36.1%	12.7 gm	68.8%	14.2 gm	52.9%	0.9
range	11.8-19.1	6-93%	8.7-17.8	9-99%	9.6-18.5	12-94%	0.7-1.2
s	1.7	19.9	2.1	19.1	1.8	19.2	0.1
$s_{\bar{x}}$	0.2	1.8	0.2	1.7	0.2	1.8	0.0
CV	10.8%	55.1%	16.5%	27.8%	12.7%	36.3%	11.0%
r	0.16		0.19		-0.08	0.030	
in % of H68	40.1%		70.0%		76.0%		
H68	90%		92%		69%		

SUMMARY AND CONCLUSIONS

Variations in field germination percentages were observed among several entries of field, sweet and supersweet corn in 3 months. Some reduction in germination percent occurred in the month of January with better germination in February and March. The decreased germination percent during January is probably due to the adverse weather conditions. In general the viability of supersweet corn seeds were low relative to sweet and field corn whether planted in the summer or winter, or when test germinated in the incubator. Therefore, selection for high and low viability were undertaken in this study.

The successful improvement of viability during 2 or 3 cycles of recurrent selection for high field viability in both shrunken-2 and brittle-2 populations was demonstrated in this investigation. The use of a modified ear to row procedure with full-sib pedigree selection proved to be effective in the selection of genotypically superior individuals. Exposure of semi-lethal genes (such as sugary-1) by the $\underline{sh}_2$ is suggested to influence viability.

Selection schemes I (half-sib 1 on 1+ with pedigree selection) and II alternating half-sib 1 on 1+ and selfing with pedigree selection) were equally effective in improving seed viability. However, seedlings developed through scheme II were very weak, therefore, improvement of germination percent through scheme I is recommended. One cycle of selection with 25% selection and with careful planting of 4 seeds/hill for 25 hills, would largely improve germination.

Selection response in a 2 cycle study to lower germination was ineffective. Phenotypic characters that may correlate with genotypic traits and therefore enhance selection procedures were researched. Neither kernel weight nor kernel density were positively correlated with germination percent.

Further research is needed to reveal other effects accompanied by the improvement of germination such as storage effect on the longevity of the seed. One must anticipate that the loss in viability of collapsed seeds might be greater in the tropics where high humidity and high temperatures prevail throughout the year. The necessity, then, of selecting for one or more generations depends on the degree of degeneration and available storage conditions. Some inbred lines and hybrids of both field and sweet corn are known to keep their viability in normal storage conditions for a very long time. Conversions of the high-sucrose genes into these lines should be considered. The incorporation of high-sucrose genes into these lines may eliminate the necessity for frequent selection to concentrate favorable genes for high germination.

CHAPTER TWO

ORGANOLEPTIC STUDIES OF HIGH SUCROSE MUTANTS

INTRODUCTION

Poor retention of flavor quality in sweet corn after harvest has been a major reason for its lack of international acceptance. The rapid conversion of soluble sugars to polysaccharides after harvest in sweet corn has long been a problem in maintaining its marketing quality. Quantitative data available in the literature show a depletion of 40 to 60% of sucrose originally present in sweet corn (sugary-1 gene) after 24 hours of storage at 25°C (Appleman and Arthur, 1919; Culpepper and Magoon, 1924). The rapid deterioration in quality is only partially reduced by storage at low temperatures.

Laughnan (1953) demonstrated that the collapsed seed mutant shrunken-2 ($\underline{sh}_2$) was very sweet at harvest by virtue of its high content of sucrose and reducing sugars. Brewbaker (1971) also reported that the sweetness of Hawaiian Supersweet #4 (sh_2) would be high between 18 and 27 days after silking, and could be harvested commercially much later than sweet corn.

Mutants superficially similar to $\underline{sh}_2$ were described by Teas and Teas (1953) as brittle-1 ($\underline{bt}_1$) and brittle-2 ($\underline{bt}_2$). Each was found to have elevated sucrose and reducing sugars in mature kernels similar to that of $\underline{sh}_2$ (Cameron and Teas, 1954). Loss of sucrose in these three genotypes is comparatively slow after harvest, which is attributed by Dickinson and Preiss (1969) to the low levels of the starch converting

enzyme, adenosine diphosphate-glucose (ADPG) pyrophosphorylase.

Since corn carrying those three mutant genes, usually referred to as "supersweet", shows great potential in areas where ordinary sweet corn is not acceptable because of its rapid loss of quality after harvest, the following studies on quality and consumer acceptance of supersweet corns were initiated. Differences in pre- and post-harvest qualities and the effects of backgrounds on the three high-sucrose mutants were examined. New high-sucrose corn products (such as "raisin corn" and freeze dried corn) were produced and evaluated. Raisin corn is produced in the following way: kernels of high-sucrose corn were cooked for 10 minutes in boiling water and oven dried at 65°F for 3 hours to bring the moisture of the kernels down to 30%.

LITERATURE REVIEW

During the past several years a considerable number of mutant genes in corn have been studied with respect to their effects on various carbohydrates in the kernel. Some of these effects are important to the effort to obtain corn starch of high amylose content (Dvonch et al., 1951); others are of interest as a means of improving the eating quality of sweet corn (Cameron and Cole, 195).

Commercial sweet corn is homozygous for the recessive gene sugary-1 ($\underline{su}_1$), which conditions the occurrence of much sugar and water soluble polysaccharides (WSP), but little starch during eating stages. Field corn carries the dominant allele ($\underline{Su}$) and contains about 74.9% total carbohydrate at 20 days after pollination (eating stage), of which 3.5% was sucrose, 2.4% reducing sugars, 2.8% WSP and 66.2% was starch (Creech, 1968).

The sugary gene, designated as ($\underline{su}_1$), located on the 4th chromosome, was described by East and Hayes (1911) and Eyster (1934). The mutant $\underline{su}_1$ gene gives a translucent, wrinkled and glassy-texture appearance to the kernels. Sugary-1 is associated with significant high endosperm content of water soluble polysaccharides (WSP) consisting largely or exclusively of phytoglycogen (Culpepper and Magoon, 1924, 1927; Dvonch et al., 1951; Laughnan, 1953; Holder et al., 1974).

Several other genes such as shrunken-2 ($\underline{sh}_2$), brittle-1 ($\underline{bt}_1$) and brittle-2 ($\underline{bt}_2$) can also change the carbohydrate balance at maturity or during kernel development. The gene shrunken-2 ($\underline{sh}_2$) located on chromosome 3, was discovered by Mains (1949). This gene is closely

linked with the a_1 locus for aleurone color. Total starch in the endosperm is significantly reduced, thus it forms a collapsed or shrunken kernel at maturity. The shrunken kernels are usually flatter, somewhat broader and frequently shallower than the non-shrunken kernels. At milk stage, the kernels are larger than normal ones and very sweet and watery.

The two genes known as brittle-1 ($\underline{bt}_1$) and brittle-2 ($\underline{bt}_2$) also have effects similar to shrunken-2 on the kernels. The gene $\underline{bt}_1$ was first described by Mangelsdorf (1926) while $\underline{bt}_2$ was first described by Teas and Teas (1953). Although these two mutants are independent simple recessives, located respectively on chromosome 5 and 4, their gross phenotypic effects are very similar; each produces a superficially shrunken endosperm of brittle texture, slightly darker than normal and intermediate in translucency between normal and $\underline{su}_1$ (Cameron and Teas, 1954). Under field conditions, the germination is very poor as compared to normal corn.

High levels of sugars and WSP are important factors of quality in sweet corn (Gonzales et al., 1972), with sugars principally affecting sweetness and WSP affecting texture (Huelsen, 1954). Total sugars and WSP of sweet corn ($\underline{su}_1$) are much higher than those of normal corn. Creech (1968) found that $\underline{su}_1$ corn had approximately 15.6% total sugars and 22.8% WSP at 20 days after pollination (DAP) as opposed to 5.9% total sugar and 2.8% WSP in normal corn. Similar results were recently reported by Gonzales et al. (1972) in the commercial sweet corn, Victory Golden, Golden Cross Bantam and Early Sunglow. They also reported a unique case of sweetness of the $\underline{su}_1$ gene in an Illinois 677a inbred

background. From 19 to 27 DAP, the sugar content of Illinois 677a is similar to that of the $\underline{sh}_2$ lines, "Illinois-Xtra-Sweet", and both show about twice the sugar content of the 3 commercial sweet corns.

Laughnan (1953) reported that $\underline{sh}_2$ kernels were extremely sweet by virtue of their high level of sugars. It was found that almost 20% of the dry weight of the shrunken kernels was composed of sugars, about a 10-fold increase over standard dent kernels. Most of the sugars consisted of sucrose, which accounted for 16% dry weight. Amount of starch was somewhat less than in $\underline{su}_1\underline{Sh}_2$, but there was only 1.6% WSP. The double recessive $\underline{su}_1\underline{sh}_2$ had even higher sugar and only 7.7% starch with only 1.8% WSP. The increased level of sugars in $\underline{sh}_2$ kernels is further evidenced by Creech's data (1968), in which he found that at 20 DAP, $\underline{sh}_2$ kernels had 4.4% WSP and 34.8% total sugar, 29.9% of which was sucrose.

Kientz et al. (1965) reported that $\underline{sh}_2$ cultivars maintained higher total sugar levels than $\underline{su}_1$ cultivars at late stages of kernel development (23 days after pollination). As the ear matured, the percentage of reducing sugars decreased rapidly in both $\underline{su}_1$ and $\underline{sh}_2$ but the sucrose percentage in $\underline{sh}_2$ remained relatively constant. Fructose, which is sweeter than both sucrose and glucose, decreased faster than glucose in both $\underline{sh}_2$ and $\underline{su}_1$. These high-sugar corns taste much sweeter and retain their sweetness much longer than sweet corn of sugary-1 type. The term "supersweet" is applied to describe the high-sugar corns released by the University of Hawaii (Brewbaker, 1971).

The developmental effects of $\underline{bt}_1$ and $\underline{bt}_2$ on a background of $\underline{Su}$ were reported by Cameron and Teas (1954). Each $\underline{bt}$ gene increased sugar

content and reduced starch content at mid-development and beyond, but no appreciable amount of WSP was ever present. The homozygous combination of $\underline{su_1}\,\underline{bt_1}$ was tested at maturity. Total sugar was higher than in $\underline{su_1}$ and starch was much lower, but there was still essentially no WSP. Brittle-2 kernels retained high levels of reducing sugars at late harvest stage better than either $\underline{bt_1}$ or $\underline{sh_2}$.

Summers and Somer (1944) and Hassid and McCready (1941) reported that the phytoglycogen (WSP) has an average chain length of 12 glucose molecules. By careful isolation, using mecuric chloride to prevent enzyme activity, Greenwood and Das Gupta (1958) and Black et al. (1966) demonstrated that the WSP fraction consisted of one type of polysaccharide, phytoglycogen. Previous workers had reported two types of branched molecules. The basal chain length was determined to be 14 glucose units by periodate oxidation.

Phytoglycogen is known as an important reserve polysaccharide of the sugary-1 genotype of maize. However, it has been shown that phytoglycogen is not found in certain mutants ($\underline{sh_2}$, $\underline{bt_1}$, $\underline{bt_2}$) containing the $\underline{su_1}$ gene, yet accumulates in certain other genotypes in which this gene is not present (Black et al., 1966). Frydman and Cardini (1964, 1965) have reported a soluble enzyme in sweet corn that transfers glucose from adenosine diphosphate-glucose (ADPG) but not from uridine diphosphate glucose (UDPG) to phytoglycogen and amylopectin.

Cameron (1947), in a study of the effect of $\underline{su_1}$ and dull ($\underline{du}$), suggested that these genes might be controlling certain enzymatic steps in polysaccharide synthesis. According to his hypothesis, the $\underline{su_1}$ locus would affect the availability of short glucose chains needed for further

synthesis of starch. But experimental evidence for this is lacking. If the normal pattern of synthesis is sucrose → WSP → starch, with the conversions proceeding so that very little of the WSP fraction is present at any time, then $\underline{su}_1$ may act to cause a partial block between WSP and starch, with resulting accumulation of WSP. It is also clear that in normal kernels WSP does not occur at all in the forms in which they are found in $\underline{su}_1$. They evidently arise in $\underline{su}_1$ by an alternate reaction such that sucrose $<^{\text{starch}}_{\text{WSP}}$. In either case $\underline{bt}_1$ and $\underline{bt}_2$ in the absence of $\underline{su}_1$ could block the utilization of sucrose at an earlier point causing sucrose to accumulate without accompanying WSP. A similar hypothesis was put forth by Laughnan (1953) who concluded that $\underline{sh}_2$ preceded $\underline{su}_1$ in synthesis of starch and WSP.

Tsai and Nelson (1966) have reported that $\underline{sh}_2$ and $\underline{bt}_2$ mutants contained no detectable ADP-glucose pytophosphorylase in the endosperm. The studies of Vidra and Loerch (1968) also indicated a lack of this enzyme in $\underline{sh}_2$. Kernels of these two defective mutants accumulate only 25% as much starch as normal ones. Tsai and Nelson concluded that the major pathway of starch synthesis occurred via the ADP-glucose pyrophosphorylase reaction. However, in the Dickinson and Preiss (1969) studies, their results definitely indicated the presence of significant but low amounts of ADP-glucose pyrophosphorylase in the endosperm of $\underline{sh}_2$ and $\underline{bt}_2$ maize kernels, 12% and 17% of the normal ones respectively. Dickinson and Preiss's data further showed that $\underline{sh}_2$ affects ADP-glucose pyrophosphorylase activity in the endosperm but not in the embryo, while in $\underline{bt}_2$ this enzyme activity appeared to be affected in both embryo and endosperm. Weaver et al. (1972), studying the ADP-glucose

pyrophosphorylase isozymes of normal, $\underline{bt}_2$, and $\underline{sh}_2$ endosperms of maize, suggested that the $\underline{sh}_2$ gene may be regulatory in nature (changing isozyme activity) and that $\underline{bt}_2$ may be a structural gene (an altered electrophoretic mobility).

Jennings and McCombs (1969) studied phosphorylase activity of the $\underline{sh}_2$ kernels. Their results indicated that $\underline{sh}_2$ locus either controlled or influenced indirectly the degree of phosphorylase activity in maize. They concluded that the high phosphorylase activity, high sucrose content and low starch conetnt of $\underline{sh}_2$ lines might indicate a role of starch degradation for phosphorylase in these genetic lines leading to sucrose accumulation. More information is needed before an adequate interpretation of the role of phosphorylase in the developing maize kernel can be made.

Cox and Dickinson (1971) observed a higher level of hexokinase in developing $\underline{sh}_2$ endosperms than in the starchy ones. In a later study (1973) they pointed out that the carbohydrate metabolism in $\underline{sh}_2$ endosperm may be altered by increased hexokinase. One consequence could be a more rapid formation of hexose phosphate which would give rise to UDP-glucose and then sucrose. In this case the increased sucrose in $\underline{sh}_2$ would result from both the blockage in the ADP-glucose formation and the enhance capacity to form precursors. A positive feedback mechanism may be operating if enhanced sucrose content (due initially to reduced ADP-glucose) causes increases in hexokinase level, which in turn, causes increased sucrose formation.

Starch biosynthesis in corn endosperm is regulated by either ADP-glucose or UDP-glucose pyrophosphorylase activity. The enzyme is

inhibited by inorganic phosphate (Dickinson and Preiss, 1969). Studies of the conversion of sucrose to starch indicate that pyrophosphate is the most effective agent in maintaining the amount of sucrose after harvest. Amir et al. (1971) have shown that the conversion of sucrose to starch in sweet corn can be regulated by pre-harvest treatments with pyrophosphate (PPi). The effect of PPi on the system is associated with inhibition of ADP-glucose synthesis, the main glucosyl donor for starch synthesis.

Amir and Cherry (1972) in further studies reported that application of ethylenediamine tetraacetic acid (EDTA) to detached ears of sweet corn had no effect on conversion of sucrose to polysaccharides, but when applied as a spray on the plants or injected into the ears, sucrose content of the kernels at harvest increased 67% while the amount of reducing sugars was doubled. They attributed this to be due to changes in cell membrane permeability. In corn kernels several layers of cells separate the vascular bundle from the endosperm tissue at the edge of the kernel (Esau, 1965). EDTA might increase the membrane permeability of those cells in separation layer, thereby increase the translocation of sucrose through this barrier. Maintenance of sweet corn quality at post-harvest would be possible as suggested by Gonzales et al. (1974) in the new inbred Illinois 677a with a $\underline{su}_1$ background which appears to give the desirable combination of high sugars and high WSP.

The first commercial high-sugar hybrid, Illini Chief, was introduced by Laughnan (1953). Two commercial $\underline{sh}_2$ hybrids are now marketed by the Illinois Foundation Seeds Corp. Several composite and synthetic

high-sugar corns adapted to the tropics have also been introduced by Brewbaker (1971). An intensive breeding program of $\underline{sh}_2$ hybrids is currently under way at the University of Hawaii.

The high-sucrose properties of both $\underline{bt}_1$ and $\underline{bt}_2$ were characterized by Cameron and Teas (1953). There are no reports of commercial or experimental breeding with $\underline{bt}_1$ and $\underline{bt}_2$ other than in Hawaii, but they appear to be promising types of supersweet. A $\underline{bt}_1$ composite has been available since 1970, and $\underline{bt}_2$ composite has recently been released by Dr. Brewbaker.

Quality of sweet corn and supersweet corn is controlled by many factors such as sweetness, tenderness and texture of the kernel, all of which constitute the desirable flavor of corn. In recent years, factors and compounds which affect the flavor of sweet corn have been studied, but essentially with no reference to the high-sucrose corns.

Correlation coefficients between sugar content and flavor as measured by taste panel scores (Winter, 1955) were higher when the corn was relatively low in sugar. When the sugar level was high, other variables became more important in determining the relative palatability of the samples tested.

Kiribuchi and Yamanishi (1963) identified dimyethyl sulfide (DMS) as a flavor component of green tea and S-methyl methionine sulfonium salt (MMS) as its precursor. The DMS and MMS were also identified in sweet corn by Bills and Keenon (1968) who reported that the DMS levels in processed sweet corn varied from 5.7 to 14.2 ppm, while frozen sweet corn when heated 10 minutes in the autoclave, produced only 0.3 to 6.8 ppm. They also found that DMS concentrations in commercially canned

sweet corn did not increase with additional heat processing. Williams et al. (1972) demonstrated that commercially canned sweet corn contained 10.1 to 16 ppm DMS. These concentrations greatly exceed the reported flavor threshold value of 12 ppb (Patton et al., 1965) and odor threshold of 0.33 ppm (Guadagni et al., 1963) for DMS in water. Very recently Williams et al. (1973) reported variations in DMS from 10.7 to 40.4 ppm among 21 blanched sweet corn hybrids chromatographically analyzed. MMS was found in unblanched corn, but no DMS was detected.

MATERIALS AND METHODS

Nine seedstocks for taste panel studies were grown at Waimanalo Farm of the University of Hawaii in April, 1973. Planting was staggered in hopes of having all cultures ready for pollination at the same time. Each of the 9 lots was planted in long rows, 180 feet in length, with approximately 250 plants per row. Of the 3 sweet corn lots, two seeds per hill were sown for sweet corn, but 4 to 5 per hill for the supersweets because of their low field emergence.

All ears were covered before silking. After anthesis, approximately 20 tassels were bagged in each of the 8 long rows and ears were cut back and hand pollinated two days thereafter. H68 did not silk at the appropriate time and therefore had to be forced. Some plants (c. 30%) were severely infected with Physoderma stalk rot. Only those ears which were hand pollinated were harvested for the taste panel. Approximately 60 to 70 ears were hand sib-pollinated in each seedstock (47 for H68). Field corn was made by crossing $\underline{bt}_2$ COMP 1 x HS ($\underline{su}_1$).

At 18 DAP, half of the 60 crosses were harvested early in the morning from the 9 seedstocks. The remaining 30 ears were harvested at 23 DAP, to constitute the second harvest. Upon harvest, ears were placed in plastic bags with crushed ice and immediately transferred to the campus of the University of Hawaii. Half of the 30 ears from each stock were stored for one week at 54°F and the remaining half was husked. Ten representative ears (well filled) were chosen from each lot and were cut into two equal halves. One half of each ear was labeled with colored plastic sticks (each color representing a seedstock) and was

prepared for the morning replication. The remaining half was labeled (with different colored sticks) and stored for 3 hours in the refrigerator for the second replication.

The 10 corn samples from each of the 9 lots were steam-cooked for 10 minutes and immediately served to 10 judges of a most diverse background (Filipino, Chinese, Caucasian, Malaysian, African, Hawaiian, Korean, German and Japanese). Subjective judgements were made of sweetness, tenderness and flavor of the corn samples with a scoring scale ranging from 1 (excellent) to 5 (very poor).

The remaining samples were stored in the refrigerator and steam-cooked in the afternoon, served to the same judges in the same manner. Ears that were stored for one week and those that were harvested at 23 DAP were prepared in the same manner. Statistical analyses of data were computed on an IBM 360 computer.

Studies of freeze-dried corn

Four seedstocks closely related to Hawaiian Sugar and CM104 were used in this experiment. The four genotypes, $\underline{bt}_1$, $\underline{bt}_2$, $\underline{sh}_2$, and $\underline{su}_1$ had been developed by conversion of the tropical corn variety, Hawaiian Sugar, and the inbred field corn, CM104. The stocks generally involve 3rd or 4th backcrosses to Hawaiian Sugar or CM104.

Four genotypes from each of the two backgrounds, plus H68, were planted to permit simultaneous harvest on two dates in August 1973, one at 18 DAP and the other at 23 DAP. Each of the genotypes was planted in four short rows, 7 feet in length. Prior to silking, mosaicked plants were rogued out (largely in CM104) and the remaining ears of

healthy plants were covered with glassine shoot bags. A sizable portion of the silk was allowed to emerge before sib-pollinating at 2 different dates. H68 and HS were pollinated earlier than CM104. Two rows were harvested from each genotype on September 30, and the remaining 2 rows were harvested on October 3, 1973, to constitute the first and second harvests.

Five ears from each of the four seedstocks were cooked in boiling water for 10 minutes and immediately transferred to a cool water container. The remaining 5 ears from each genotype were left raw. Kernels were removed by hand or with the aid of a knife, depending upon the condition of the kernels (for example, big kernels were easy to remove by hand in order to preserve the whole kernel). Kernels were frozen overnight in aluminum trays and then transferred to a Virtis freeze-drying unit. Freeze-drying was carried out at a vacuum of 0.10 $\pm$ 0.05 mm Hg. shelf temperature at 140°F for about 16 hours. The final freeze-dried products were vacuum-packed in multilayer moisture-water-proof pouches and stored at room temperature until February 20, 1974.

Several hours before the taste panel test was scheduled (February 20, 1974), a desired amount of sample (35-40 gm.) was removed from each package and placed in a bottle, while the remaining sample was re-packed under vacuum. Chaffs or glumes, broken and collapsed kernels were removed and the remaining clean product was weighed. Three gram samples were served in one ounce paper cups for 3 consecutive days (as 3 replications) to a panel of 10 judges. Two additional ears were used for the determination of dry matter, protein, and pericarp thickness from each lot (Appendix Table 1). Both BLSD (Duncan, 1965) and DLSD (Anonymous, 1969) were used for ranking the seedstocks.

RESULTS

1. Effect of harvesting date and storage on 9 vegetable corns related to Hawaiian Sugar

Tenderness, sweetness and flavor ratings on a scale of 1 (excellent) to 5 (very poor) were made by taste panels on 9 seedstocks of sweet and supersweet corns (Table 8). A complete factorial experiment consisting of 2 harvest dates and 2 storage periods was conducted, with organoleptic ratings provided by a 10-member panel. Ears were harvested at 18 days (early mature) and 23 days (late mature), and served fresh or stored (7 days at 54°C) prior to evaluation. All evaluations were made in 2 replicates, one served in the morning, and one in the afternoon.

The average ratings of the 10 judges, from duplicate runs, varied widely, rarely better than 2.0 (highly desirable). The sample most consistently scored in this study was the commercial sweet corn hybrid H68 (Brewbaker, 1968). The panel averages for this hybrid were 3.1 (tenderness), 4.5 (sweetness) and 4.1 (flavor), reflecting rather critical attitudes of the panelists. The field corn control ($\underline{bt}_2 \times \underline{su}_1$) ranked somewhat lower. The data appeared to normalize about means of approximately 3.0 for fresh corn and 3.7 for stored corn (Table 8).

Data are summarized in Tables 8 and 9 in two ways to show differences between fresh and stored ears (Table 9), and between samples harvested at 18 DAP and at 23 DAP (Table 10). Almost universally the supersweet samples were marked superior in tenderness and flavor, as well as in sweetness, irrespective of the date of harvest or post-harvest

Table 8. Average scores* of 10 judges and 2 reps for tenderness, sweetness and flavor of 9 seedstocks related to Hawaiian Sugar (HS).

Seedstock	Harvested 18 DAP						Harvested 23 DAP					
	No storage			Stored 7 days			No storage			Stored 7 days		
	Tdr.	Swt.	Flv.	Tdr.	Swt.	Flv.	Tdr.	Swt.	Flv.	Tdr.	Swt.	Flv.
$\underline{bt}_1$ COMP 2	2.35	1.80	2.25	3.20	3.05	3.35	2.70	2.05	2.75	3.30	3.40	3.25
$\underline{bt}_2$ COMP 1	2.70	1.80	2.60	2.85	2.50	2.70	3.00	2.20	2.95	3.45	2.30	2.75
$\underline{fl}_2\underline{sh}_2$ COMP 1	2.35	2.15	2.20	3.60	3.90	3.90	2.85	2.35	2.65	3.65	3.45	3.30
$\underline{sh}_2$ Syn 2	2.65	2.30	2.10	3.15	4.25	3.80	2.65	2.15	2.75	3.20	3.45	3.35
$\underline{sh}_2$ COMP 2g	3.00	2.75	2.75	3.15	3.30	3.80	3.40	3.20	2.95	3.80	4.00	3.80
H68	2.60	4.10	3.75	3.85	4.80	4.60	2.35	4.05	3.65	3.50	4.70	4.40
HS	3.50	3.90	3.55	3.55	4.35	3.40	3.75	4.05	3.75	3.80	4.85	4.50
$\underline{o}_2\underline{su}$ COMP 1	3.50	4.15	3.65	3.50	4.60	4.50	3.90	4.15	4.00	3.80	4.80	4.50
($\underline{bt}_2$ x $\underline{su}_1$)	4.55	4.70	4.55	4.05	4.85	4.65	4.60	4.95	4.70	3.75	4.80	4.55
Averages	3.02	3.01	3.04	3.43	3.96	3.85	3.24	3.24	3.35	3.58	3.97	3.82

*Scoring scale: 1 (excellent) to 5 (very poor).

Table 9. Average scores* of 10 judges, 2 harvests and 2 reps for tenderness, sweetness and flavor (based on Table 8).

Seedstock	Tenderness		Sweetness		Flavor	
	Fresh	Stored	Fresh	Stored	Fresh	Stored
$\underline{bt}_1$ COMP 2	2.5 a	3.3 cde	1.9 a	3.2 bc	2.5 a	3.3 cd
$\underline{bt}_2$ COMP 1	2.9 abc	3.1 bcd	2.0 a	2.4 a	2.8 bc	2.7 bc
$\underline{fl}_2\underline{sh}_2$ COMP 1	2.6 a	3.6 def	2.3 a	3.7 cd	2.4 a	3.6 d
$\underline{sh}_2$ Syn 2	2.7 ab	3.2 cde	2.2 a	3.9 bc	2.4 a	3.6 d
$\underline{sh}_2$ COMP 2g	3.2 cde	3.5 def	3.0 b	4.1 de	2.9 bc	3.8 de
H68	2.5 a	3.7 def	4.1 d	4.8 f	3.7 de	4.5 f
HS	3.6 def	3.7 def	4.0 d	4.6 ef	3.7 de	4.2 ef
$\underline{o}_2\underline{su}$ COMP 1	3.7 def	3.7 def	4.2 de	4.7 f	3.8 de	4.5 f
($\underline{bt}_1$ x $\underline{su}_1$)	4.6 f	3.9 ef	4.8 f	4.8 f	4.6 f	4.6 f
DLSD @ 0.01	0.38		0.36		0.40	

*Scoring scale: 1 (excellent) to 5 (very poor).

Table 10. Average scores* of 10 judges, 2 storage regimes and 2 reps for tenderness, sweetness and flavor of corn harvested at 18 DAP and 23 DAP (based on Table 8).

Seedstock	18 DAP	23 DAP	18 DAP	23 DAP	18 DAP	23 DAP
$\underline{bt}_1$ COMP 2	2.8	3.0	2.4	2.7	2.8	3.0
$\underline{bt}_2$ COMP 1	2.8	2.9	2.1	2.3	2.7	2.9
$\underline{fl}_2$ $\underline{sh}_2$ COMP 1	2.9	3.3	3.0	2.9	3.0	2.9
$\underline{sh}_2$ Syn 2	2.9	2.9	3.3	2.8	2.9	3.1
$\underline{sh}_2$ COMP 2g	3.1	3.6	3.0	3.6	3.3	3.4
H68	3.2	2.9	4.4	4.4	4.1	4.0
HS	3.5	3.8	4.6	4.4	3.5	4.1
$\underline{o}_2\underline{su}$ COMP	3.5	3.9	4.4	4.5	4.1	4.3
($\underline{bt}_2$ x $\underline{su}_1$)	4.3	4.2	4.7	4.9	4.6	4.6

*Scoring scale: 1 (excellent) to 5 (very poor).

storage.

Storage for one week made the conventional sweet corns (H68, HS and $\underline{o_2 su}$ COMP 1) essentially unpalatable to the judges, who ranked them as badly as field corn ($\underline{bt_1}$ x $\underline{su}$) following storage (Table 9, Figure 9). In contrast, $\underline{bt_1}$ and $\underline{bt_2}$ high-sucrose stocks showed no major loss of sweetness or flavor in storage (Table 10). The $\underline{bt_2}$ COMP 1 was exceptional in that a week's storage had no effect on taste panel evaluations. Recognizing that the base for comparisons after a week's storage was a poorer product, it is nonetheless certain that the $\underline{bt_2}$ genotypes have shown superior quality retention in all evaluations.

The differences between harvest dates were very small, with no significant loss of quality on the average for any parameter (Table 10). The conditions during maturation had been excellent, with good light and adequate moisture, and 18-19 days would represent peak commercial harvest. The loss in tenderness and sweetness in the following 5 days was less than expected, and many seedstocks showed no change at all.

Analysis of variance confirmed the apparent significance of most main effects (Table 11). The seedstock differences were highly significant in all parameters measured. Storage effects were generally significant, as were differences among the judges. As observed previously (cf. Table 10), the effects of harvest date were not generally significant, nor was there a significant interaction of seedstocks and harvest date. The effect of harvesting date on tenderness for 5 supersweet corn stocks was somewhat variable. Shrunken-2 COMP 2g was the only stock that showed a major increase in toughness as a function of harvest date (Table 10), although increased

Figure 9. Storage effect on tenderness, sweetness and flavor. Seedstocks: 1(H68), 2(HS), 3($\underline{o}_2\underline{su}_1$ COMP 1), 4($\underline{bt}_1$ COMP 2), 5($\underline{bt}_2$ COMP 1), 6($\underline{sh}_2$ Syn 2), 7($\underline{sh}_2$ COMP 2), 8($\underline{fl}_2\underline{sh}_2$ COMP 1) and 9($\underline{bt}_2$ x $\underline{su}_1$).

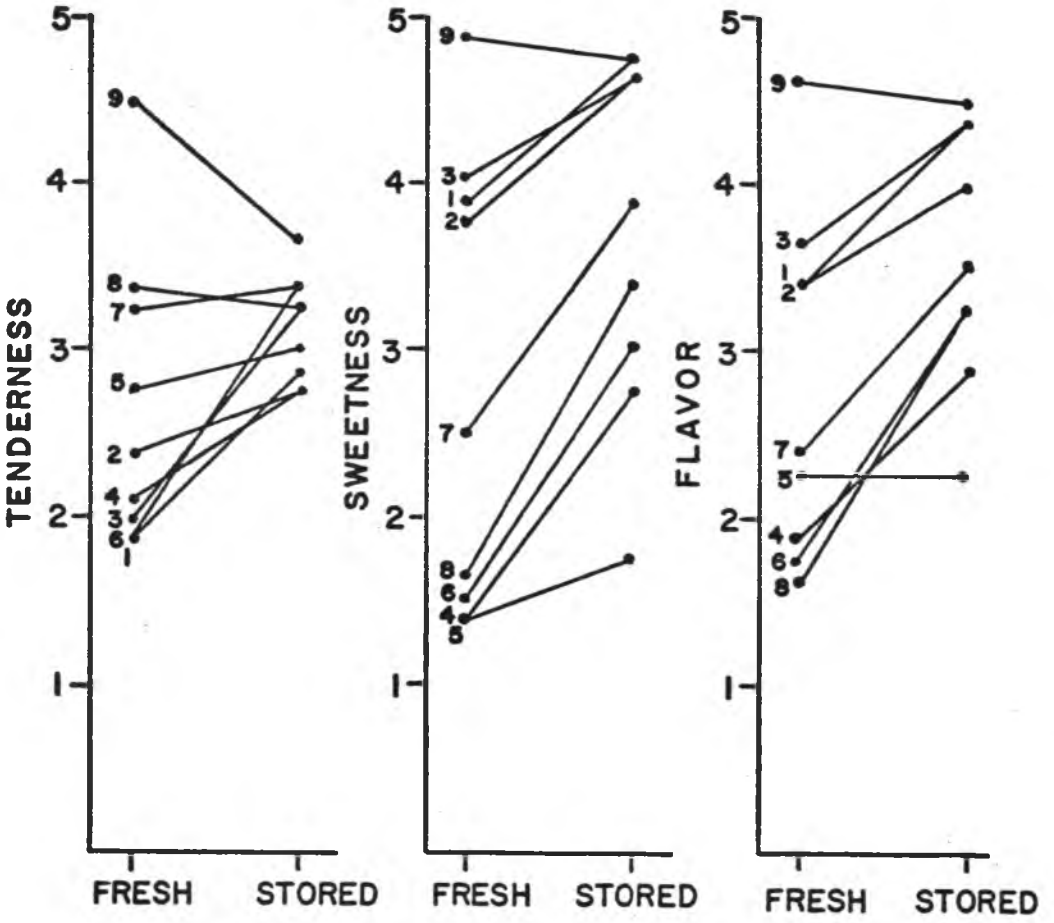

TENDERNESS
SWEETNESS
FLAVOR
5
4
3
2
1
FRESH
STORED

Table 11. Analysis of variance for average taste panel scores in Table 8.

Source	df	Tenderness	Sweetness	Flavor
Seedstocks (SS)	8	16.3**	73.7**	31.2**
Storage (S)	1	23.8*	117.6**	82.7*
Harvest (H)	1	6.2**	1.3	1.3
Judges (J)	9	10.8**	8.1**	11.3**
SS x S	8	7.2**	4.6**	4.2**
SS x H	8	1.7	0.5	0.6
SS x J	72	1.6	1.0	1.1
S x H	1	0.1	0.9	6.1**
S x J	9	2.8**	2.7**	4.1**
H x J	9	1.5	1.3	2.7**
SS x S x H	8	0.3	0.9	1.0
SS x H x J	72	0.8	0.7	0.9
S x H x J	9	0.9	1.1	1.1
SS x S x J	72	1.1	0.6	0.9
Reps	1	0.001	0.013	1.606
Error	431	0.76	0.68	0.84

Error includes rep interactions and SS x S x H x J.

toughness of the kernels was expressed by most supersweet stocks as the result of one week storage.

The judges showed significant differences in their rating scales, with only one interaction that was significant, storage x judge (Table 11). Some judges were evidently much more critical in their appraisal of the stored ears, while others were less critical (Appendix Tables 2 and 3). These differences in judgment did not extend to the seedstocks, as witnessed by non-significant SS x J and SS x S x J variances. No higher order interactions were significant.

It is interesting to note that the different ethnic background of panelists may account for differences in their judgments of high sucrose and sweet corn stocks. In general, all judges preferred the high sucrose types in all qualities scored. The Caucasians showed greater preference for early harvests, whereas Africans, Asians and Filipinos, in particular, preferred later harvests when kernels were maximally filled.

One judge expressed exceptional preference for $\underline{fl_2 sh_2}$ COMP 1b in 3 parameters (no storage) (Appendix Table 2, Judge #10). This exceptional preference might be due to the fact that this double mutant is rich in methionine which acts as a precursor for dimethyl sulfide, a major flavoring compound in sweet corn (Williams, 1973).

Two basic questions emerged from the results of this study: why did the high sucrose corns have better flavor, and why were they more tender than the sweet corn genotypes, particularly at the late harvest stage? Significant correlations occurred between sweetness and flavor, sweetness and tenderness, and tenderness and flavor (Table 12).

Table 12. Regression and correlation analysis of average taste panel scores in Table 8.

Conditions	Parameter	Sweetness vs Flavor	Flavor vs Tenderness	Sweetness vs Tenderness
Fresh	b	1.38	0.74	0.47
	a	-1.24	0.76	1.65
	r	0.96**	0.82*	0.75*
	R	0.92**	0.67*	0.56*
Stored	b	1.21	0.36	0.28
	a	-0.71	2.14	2.41
	r	0.96**	0.88**	0.86**
	R	0.92**	0.77**	0.74**

The interrelationships of flavor and sweetness are also supported by the observations of Moskowitz (1974) who reported that the sensation of "sweetness" increased directly with concentrations of sucrose, whereas "pleasantness" at first increased, then decreased. Moskowitz reported the breakpoint for maximum pleasantness to be at the 18% glucose. The correlation between sweetness and tenderness is a secondary one. Tenderness of supersweet corn is probably attributed to the fact that less starch is being synthesized in the endosperm; later studies of pericarp thickness appear to confirm this (Chapter 4).

2. Quality and sensory evaluations of 8 vegetable corn seedstocks

A second organoleptic experiment involved ears of corn from 8 seedstocks related to Hawaiian Sugar, harvested 19 DAP. The ears were steam-cooked and served to 20 judges, with two reps in one day. Average scores of the 20 judges in duplicate for tenderness, sweetness and flavor are summarized in Table 13.

Variance analysis (Table 14) revealed significant differences between seedstocks and between judges. No significant differences were observed between morning and afternoon sessions (reps). The interaction of seedstock x judges was not significant.

Average scores for tenderness ranged from 2.4 to 3.6. All supersweet corns were tender at 19 DAP. Sweetness scores varied from 1.0 for $\underline{bt}_1$ to 4.3 for Hawaiian Sugar. All high-sucrose corns were judged sweeter than the sweet corns. Score values for flavor ranged between 2.3 for $\underline{bt}_2$ to 4.2 for Hawaiian Sugar. Brittle-2, $\underline{sh}_2$ COMP 2, and $\underline{bt}_1$ COMP 2 were top-ranked in flavor. All sweet corns were

Table 13. Average scores* of 20 judges and 2 reps for tenderness, sweetness and flavor of 8 seedstocks related closely to Hawaiian Sugar.

Seedstocks	Tenderness	Sweetness	Flavor
$\underline{bt}_2$ COMP 1	2.55 a	2.10 a	2.30 a
$\underline{bt}_1$ COMP 2	2.87 abc	1.95 a	2.80 a
$\underline{sh}_2$ COMP 2	2.40 a	2.45 a	2.33 a
$\underline{sh}_2$ Syn 2	3.05 abc	2.55 ab	2.73 ab
$\underline{fl}_2\underline{sh}_2$ COMP 1	2.88 abc	3.15 ab	3.08 b
$\underline{o}_2\underline{su}_1$ COMP 1	3.05 abc	3.18 b	3.35 bc
H68	3.33 bc	4.00 c	3.93 cd
HS	3.58 c	4.30 c	4.18 d
DLSD @ 0.05	0.77	0.70	0.63

*Scoring scale: 1 (excellent) to 5 (very poor).

Table 14. Analysis of variance for average scores in Table 13.

Source	df	Tenderness	Sweetness	Flavor
Seedstock	7	6.00**	29.48**	22.33**
Judges	19	3.75**	4.45**	4.89**
SS x J	133	1.15	0.79	0.87
Rep	1	5.51	0.45	2.81
Error	159	1.23	1.26	1.01

Error includes rep interactions and SS x J effects.

significantly inferior in flavor to the high-sucrose corn (Appendix Table 4). This is probably due to the correlation of sweetness with flavor.

Regression analysis (Table 15) indicated a highly significant coefficient of determination between sweetness and flavor (R=86%), tenderness and flavor (R=86%) and sweetness and tenderness (R=67%).

Brittle-2 and brittle-1 were again top-ranked in most categories, even under a broader judgment of 20 panelists. Two stocks, $\underline{fl_2}\underline{sh_2}$ and $\underline{sh_2}$ Syn 2, which in the previous experiment ranked as well as $\underline{bt_1}$ and $\underline{bt_2}$, were now ranking far behind. Both $\underline{fl_2}\underline{sh_2}$ and $\underline{sh_2}$ Syn stocks deserve further experiments to explore some of their exceptional features (e.g. tenderness).

The brittle-1 stock was as sweet as $\underline{bt_2}$ at early harvest, but the latter genotype was judged superior in flavor. There appear to be other compounds beside sugars which contribute to the unique flavor of this $\underline{bt_2}$ genotype. Further investigations are required in order to determine the primary constituents of $\underline{bt_2}$ flavor. In addition, there are many more improvements to be made in $\underline{bt_2}$ COMP 1 (e.g. selection against red silk and red cob), and there is much more to be learned about its physiology (e.g. why it has better quality retention than other genotypes).

3. Flavor evaluation of 14 new supersweet hybrids

Fourteen new $\underline{sh_2}$ singlecross hybrids from Dr. Brewbaker's breeding program were evaluated for flavor, using two representative open-pollinated ears selected 18-20 DAP from each hybrid. Each of the two

Table 15. Regression and correlation of average taste panel scores in Table 13.

Parameters	Sweetness vs Flavor	Tenderness vs Flavor	Tenderness vs Sweetness
b	1.15	1.36	1.85
a	-0.60	0.52	-2.52
r	0.93	0.93	0.82
R	0.86	0.86**	0.67*

ears from the 14 hybrids was served raw to 3 judges for the evaluation of the three qualities--flavor, sweetness and tenderness--on a scale of 1 to 5 (1=best). The same ears were then gathered and steam-cooked for 8 minutes and served to the same judges who again evaluated the 3 parameters.

Average scores of 3 judges for sweetness and flavor of 14 $\underline{sh}_2$ hybrids (uncooked and cooked) are presented in Table 16. The judges showed no special preference for the cooked or the uncooked corn, either in sweetness or flavor (Table 16). Flavor scores ranged from 1.8 to 3.7 for uncooked corn, and 1.2 to 4.0 for the cooked. Sweetness scores ranged from 1.5 to 3.5 for uncooked corn and from 1.8 to 3.5 for cooked corn.

Variance analysis (Table 17) revealed highly significant differences between 14 hybrids for both sweetness and flavor. Blanching differentially tended to decrease sweetness, although some hybrids were not affected. In general, blanching improved the flavor of many hybrids, although some of them like row #4843, 4857, 4855 and 4831 deteriorated in flavor after blanching. Hybrids 4875, 4878 and 4741 were top-ranked in both sweetness and flavor.

Correlations of flavor (y) with sweetness (x) for both cooked and uncooked corn are given in Table 18. The correlations of cooked and uncooked samples were about the same (r=0.74), but the slope (b) of cooked corn was slightly steeper (b=1.28) than that of the uncooked corn (b=0.71).

The 14 $\underline{sh}_2$ hybrids in this study had been grown under undesirable winter conditions (lower light intensity, more rain), when lower

Table 16. Average scores of 3 judges for flavor and sweetness of 14 $\underline{sh}_2$ hybrids (cooked vs uncooked).

Hybrid row #73-	Average scores on a 1-5 scale (1=best)			
	Sweetness		Flavor	
	U	C	U	C
4741	1.5 a	2.3 bc	2.5 bcde	1.8 ab
4748	3.5 c	2.7 bc	3.2 cdef	2.7 bcde
4774	2.0 bc	3.0 bc	1.7 a	2.5 bcde
4800	3.3 c	2.5 bc	3.4 cdef	2.3 abcd
4831	2.8 bc	2.8 bc	2.3 abcd	3.5 def
4839	2.7 bc	2.3 bc	2.5 bcde	2.2 abc
4843	3.0 bc	2.8 bc	3.7 ef	4.0 f
4845	3.0 bc	2.8 bc	3.2 cdef	3.0 bcdef
4855	2.2 bc	3.5 c	2.8 bcdef	3.2 cdef
4857	2.7 bc	3.0 bc	2.8 bcdef	3.7 ef
4861	1.8 ab	2.2 bc	2.2 abc	2.5 bcde
4867	2.5 bc	2.3 bc	2.8 bcdef	2.5 bcde
4875	2.0 bc	2.0 bc	1.8 ab	1.2 a
4878	1.8 ab	1.8 ab	2.2 abc	1.8 ab
Averages	2.4	2.5	2.6	2.6
DLSD @ 0.05	1.29		1.42	

Table 17. Analysis of variance for average taste panel scores in Table 16.

Source	df	Mean square Flavor	Sweetness
Treat (T)	1	0.02	0.09
Hybrid (H)	13	2.21**	1.11**
T x H	13	0.68	0.52
Judges (J)	2	0.12	0.58
Error	54	0.41	0.39

Table 18. Correlation of flavor (y) with sweetness (x) for average scores in Table 16.

	Statistics of Correlation	
	U	C
b	0.713	1.281
a	0.877	-0.658
r	0.739	0.741
s_{xy}	0.382	0.507
R	55%**	55%**

quantities of sugars are synthesized and transported into the kernels. The decrease in amount of sugars (sweetness), either by blanching or undesirable winter conditions, may have facilitated the discrimination of good from bad flavors, which are usually confounded with high-sugar contents of summer-grown supersweet corn. This is in agreement with the data of Winter (1955) who reported that flavor was influenced more by variations in sugar content, when the sweet corn was relatively low in sugar, than when the sweet corn was relatively high. This indicates that when the sugar level of sweet corn is sufficiently high, other variables become more important in determining the relative attractiveness of the sweet corn samples tested.

A major fact which became evident as a result of this study is that a hybrid scored as desirable in flavor uncooked may be scored as bad in flavor after it is blanched (Table 16). Selection for quality of sweet or supersweet corn should preferably be based upon corn that has been cooked (blanched), since that is the condition of the corn when placed on the table of the consumer.

4. Effect of cooking on crispness, sweetness and flavor of four freeze-dried sweet and supersweet corns

Freeze-dried supersweet corn was observed to be puffed, to smell like fresh harvested corn and to be tender and crisp. To preserve the crispness, it had to be stored in an airtight container. Unlike the freeze-dried supersweet corn, freeze-dried sweet corn was less crisp and sticks to the teeth when chewed.

Crispness, sweetness and flavor ratings on a scale of 1 (excellent) to 5 (very poor) were made by taste panels on freeze-dried kernels of 4 seedstocks of sweet and supersweet corn (Table 19). A factorial experiment consisting of 2 cooking time and 4 genotypes was initially planned. Since one sample (uncooked brittle-2 genotype) had molded, however, the orthogonality was destroyed. Therefore, the remaining 6 samples were treated as 7 independent treatments with organoleptic ratings provided by a 10-member panel. Ears were harvested 23 DAP and panelists were served uncooked or cooked freeze-dried corn. All evaluations were made on 3 consecutive days as 3 reps.

The average ratings of supersweet corn ranged from 1.4 to 2.4 for crispness, from 1.6 to 3.2 for sweetness and from 1.6 to 3.4 for flavor. Panel averages for sweet corn hybrid H68 were 2.7, 4.6 and 4.2 for crispness, sweetness and flavor respectively. The supersweets were top-ranked in crispness, sweetness and flavor, irrespective of their cooking treatment. The sweet corn hybrid H68, however, ranked the worst in all 3 categories, either uncooked or cooked.

Cooking corn in boiling water for 10 minutes had a great effect on sweetness and flavor. The degree of sweetness was reduced significantly by cooking in the $\underline{bt}_1$ genotype (Table 19). Cooked $\underline{bt}_2$ was sweeter than the $\underline{bt}_1$; data were not obtained in this experiment of the uncooked $\underline{bt}_2$ (Table 19). No significant changes were observed in $\underline{sh}_2$ or $\underline{su}_1$ for sweetness as the result of blanching. Flavor of $\underline{bt}_1$ deteriorated with blanching in this study, but not that of the $\underline{bt}_2$ genotype. Again, $\underline{bt}_2$ appeared to be the preferred genotype, but conclusions are drawn here with no reference to the uncooked $\underline{bt}_2$. Shrunken-2 improved in flavor

Table 19. Average scores* of 10 judges and 3 reps for crispness, sweetness and flavor of freeze dried corn at 23 DAP.

Seedstocks	Crispness[z]		Sweetness[z]		Flavor[z]	
	U	C	U	C	U	C
$\underline{bt}_1$	1.60 a	1.43 a	1.66 a	2.83 b	2.23 ab	2.90 bc
$\underline{bt}_2$	----	1.53 a	----	2.00 a	----	1.96 a
$\underline{sh}_2$	2.36 b	1.70 a	3.16 b	2.83 b	3.43 d	2.56 bc
$\underline{su}_1$	2.80 c	2.53 bc	4.60 c	4.60 c	4.23 e	4.13 e
DLSD @ 0.05	0.38		0.35		0.42	

*Scoring scale: 1 (excellent) to 5 (very poor)

[z]Means followed by the same letters are not significantly different from each other.

as a result of cooking. Cooking had neither a positive nor a negative effect on flavor of H68.

Analysis of variance (Table 20) revealed highly significant differences of the genotypes for crispness, sweetness and flavor; of judges for crispness; and of genotype x judges interaction for crispness and flavor. Judges showed significant differences in their scoring for crispness (Table 20), several were far more critical in their evaluations than others. These marked differences in judgment did not extend to the other two parameters, as witnessed by non-significance of sweetness and flavor. The relatively clear-cut degree of sweetness may have made it easier for the 10-member panel to discriminate for this parameter and consequently for flavor which is correlated with sweetness. The significant interaction between judges and genotypes for crispness is not clearly interpretable. In general all the judges preferred the cooked $\underline{bt}_2$ genotype in all qualities scored.

Cooking corn in general tends to decrease sweetness of the freeze-dried product on the one hand, and improve the flavor of some genotypes on the other. Reduced sweetness is probably due to the diffusion of sugars from the kernels into the boiling water, as observed by Williams and Nelson (1973). Loss in sugars should be minimized by steam cooking, rather than by boiling in hot water. Changed or improved flavor by boiling corn is not as yet clearly understood. Williams and Nelson (1973) have attributed the improved flavor of sweet corn by cooking to be due to the high levels of dimethyl sulfide and other compounds which are lacking or at low levels in the raw corn.

Table 20. Analysis of variance for average scores in Table 19.

Source	df	Mean Squares		
		Crispness	Sweetness	Flavor
Seedstock (S)	6	9.23**	39.56**	24.10**
Judges (J)	9	7.40**	1.04	0.96
S x J	54	1.02**	0.71	1.52**
Rep	2	1.34	0.95	0.74
Error	138	0.46	0.43	0.60

Additional beneficial effects on the freeze-dried kernels derived from cooking were enhancement of the color, from a dull hue to a vivid yellow, and a differential reduction in hygroscopic character of kernels of different genotypes (Appendix Table 4). The improved color of the kernels appeared to result from the removal of air, accompanied by a replacement of moisture in the free air space. Although the mechanism of the cooked corn being less hygroscopic than the uncooked corn is not clearly understood, it is reasonable to postulate that denaturation of protein, loss in sugars and concentration of starch and its rapid gelatinization accounts for the reduced hygroscopicity of cooked kernels.

There were variations in crispness within a given sample from each genotype. The uncooked samples were slightly shrivelled. These were rather less crisp than the fully expanded kernels. Out of all the blanched genotypes, $\underline{bt}_2$ was the most successful in retaining its natural structure (rapid gelatinization of starch, more solid roof and fully expanded shape of the kernels). The crispy or crunchy nature of $\underline{bt}_2$ helps account for the excellent flavor of this genotype.

5. The effect of 4 mutant genes on freeze-dried corn quality in 2 backgrounds

Evaluation of $\underline{bt}_1$, $\underline{bt}_2$, $\underline{sh}_2$ and $\underline{su}_1$ conversions of the CM104 inbred (Brewbaker, 1972) and in Hawaiian Sugar, with H68 as a control, was made on freeze-dried kernels for crispness, sweetness and flavor (Tables 21 and 22). The four genotypes, in the two backgrounds, plus H68 sweet corn, were harvested 18 and 23 DAP. Brittle-1 samples from

Table 21. Average scores* of 12 judges and 3 reps for crispness, sweetness and flavor of corn harvested 18 DAP.

Genotypes	18 DAP Crispness HS	Crispness CM104	Sweetness HS	Sweetness CM104	Flavor HS	Flavor CM104
$\underline{bt}_2$	1.28 a	2.17 e	1.72 b	2.88 ef	1.83 a	2.53 d
$\underline{bt}_1$	1.53 b	2.06 cd	1.47 a	3.06 h	1.75 a	3.08 ef
$\underline{sh}_2$	1.53 b	2.03 cd	1.75 bc	2.11 de	2.28 bc	2.29 bc
$\underline{su}_1$	2.81 f	3.17 g	3.81 i	4.69 k	3.67 g	4.47 i
H68	2.75 f		4.08 j		3.94 h	
DLSD @ 0.05	0.23		0.23		0.25	

*Scoring scale: 1 (excellent) to 5 (very poor).

Table 22. Average scores* of 12 judges and 3 reps for crispness, sweetness and flavor of corn harvested 23 DAP.

Genotypes	23 DAP Crispness HS	Crispness CM104	Sweetness HS	Sweetness CM104	Flavor HS	Flavor CM104
$\underline{bt}_2$	2.06 cd	1.64 bc	2.64 g	1.97 cd	2.58 d	2.11 b
$\underline{bt}_1$	1.97 cd	----	2.97 h	----	3.22 f	----
$\underline{sh}_2$	2.14 e	1.86 cd	3.00 h	2.39 f	2.86 c	2.47 cd
$\underline{su}_1$	3.47 h	3.31 gh	4.69 k	4.00 h	4.50 i	4.44 i
H68	3.25 gh		4.66 k		4.47 i	
DLSD @ 0.05	0.23		0.23		0.25	

*Scoring scale: 1 (excellent) to 5 (very poor).

CM104 were recovered from the second harvest but were not enough to be included in the taste panel. The 17 remaining samples were cooked in boiling water, freeze-dried, and then treated as 17 independent treatments. In Tables 21 and 22, paired values of the ratings in backgrounds HS and CM104 are presented. All evaluations were made in 3 replications, each served every other day. The 4 genotypes in HS and the control were served for organoleptic evaluation to a 12-member panel in the morning and the 4 genotypes in CM104 were served to the same panel in the afternoon.

In general all high-sucrose corns in the Hawaiian Sugar background were rated better than those in the CM104 background. The panel ratings of hybrid H68 were 3.0 (crispness), 4.4 (sweetness) and 4.2 (flavor). This hybrid and the $\underline{su}_1$ genotype in both HS and CM104 backgrounds were generally unacceptable to the panelists (Table 21). The quality of $\underline{sh}_2$, $\underline{bt}_1$ and $\underline{bt}_2$ genotypes in the CM104 background, improved with age, whereas, a decrease in quality was found in these genotypes in the HS background at 23 DAP (Table 22).

Analysis of variance (Table 23) revealed significant differences between main effects and interaction between judges x treatments for all parameters evaluated. Differences between $\underline{bt}_2$ vs $\underline{bt}_1$, $\underline{bt}_2$ vs $\underline{sh}_2$ and $\underline{bt}_1$ vs $\underline{sh}_2$ were more or less non-significant. It should also be noted that high-sucrose genotypes at 30+ DAP in the HS and CM104 backgrounds were still impressively sweet and tender.

Table 23. Analysis of variance of data summarized in Table 21, 22.

Source	df	Mean squares Crispness	Sweetness	Flavor
Treatments* (T)	16	17.60**	48.69**	35.77**
Judges (J)	11	20.97**	5.01**	6.12**
T x J	176	1.30**	0.76**	0.88**
Rep	2	3.51**	0.87	0.04
Error	406	0.44	0.44	0.53

*"Treatments" include Genotype, Background, DAP and all other Interactions.

SUMMARY AND DISCUSSION

The potentialities that high-sucrose corn offers for the tropics and temperate regions are extensive. That they can be harvested as late as 28 to 32 DAP and still retain their high-sucrose quality gives them an added advantage over other sweet corn types. All high-sucrose HS stocks are characterized by high prolificacy. This can be of great advantage to small farmers and gardeners in the tropics, who could have a continuous harvest of corn all year round. The virtue of quality retention at delayed harvest, or after storage, is of great benefit to developing countries where transportation is hazardous during the rainy season and refrigeration is a luxury. Some high-sucrose lines are as early or even earlier than H68 in maturity (70 days). Those lines with early maturity, if selected out, could be planted as an early composite in areas where rainfall is irregular and falls for only a short duration. After the ears have been harvested, the remaining stover can be fed to animals.

The high-sucrose corn product can be used in a multitude of ways, either eaten as fresh raw corn, or boiled or steamed for 5 to 8 minutes and then consumed. The blanched ears can either be partially dried or the kernels removed and dried to produce tasty corn "raisins". Another possibility is to allow complete development of the kernels until physiological maturity and then sun dry them. This product can also be rehydrated for 24 hours and boiled (preferably before rehydration) for 5 to 10 minutes, which yields a palatable dish.

Preference of high-sucrose corn and especially $\underline{bt_2}$, by a diverse group of judges (Filipino, Caucasian, Chinese, German, Korean, Jewish, and Japanese) is a strong indication that this product will readily be accepted by peoples of developing as well as developed countries. Extracting high quality protein from cereal grains and to some extent from leaves, has been discussed by Pirie (1961). A most objectionable aroma is noticed in association with protein extracted from leaves. Attempts to mask this unpleasant odor with several fruit mixtures have failed. Most legumes are rich in protein although low in yield, and the prolonged period of cooking (3 to 5 hours) causes them to lose some of their essential amino acids (e.g. lysine) which are labile to heat (Bressani, 1972).

Cereal grains (including corn), when prepared for human consumption, are subject to many different processes, most of them designed to remove fibrous layers from the grain. However, these processes reduce the nutritional value of the grain. Traditional methods (in Africa) using a pestle and mortar usually produce a cereal grain product which has lost some of its pericarp and aleurone layer. Heavy milling often removes the germ, in addition to the pericarp and aleurone layer. The germ and aleurone are the most nutritional parts of the corn kernel (Latham, 1965).

High-sucrose corns resolve many of these problems since the entire kernel is palatable. Brittle-2 stock has about 15% protein of which 3 to 4% is lysine (Barbosa, 1971); it is high in tryptophan, has 18% lipids (Flora, 1972) and 24 to 30% total sugars (Cameron and Teas, 1954).

Delayed harvesting from 18 DAP to 23 DAP increase the dry matter content 50% (Appendix Table 5), thus increasing the caloric value without impairment to the quality or appreciable loss of sugars. No milling is necessarily required prior to the preparation of the dish. Supersweet corns in general do not require sophisticated preparations, nor do they require ingredients other than water to prepare a simple, inexpensive and highly nutritious dish. A more promising genotype is a double mutant brittle-2 opaque-2, which has all the qualities of $\underline{bt}_2$ plus an increased level of lysine (6%) (Misra et al., 1972).

CHAPTER THREE

CONVERSIONS OF DOUBLE MUTANTS IN THREE BACKGROUNDS AND ANIMAL FEEDING STUDIES

INTRODUCTION

Nutritional quality of protein in field corn can be improved by the incorporation of the opaque-2 ($\underline{o}_2$), opaque-7 ($\underline{o}_7$) or floury-2 ($\underline{fl}_2$) genes. Substitution of normal alleles by any of these three is accompanied by increased levels of lysine and tryptophane. The opaque-2 gene in particular has been widely used since 1966 and introduced into many commercial field corn varieties and hybrids.

Research on the incorporation of these high-lysine genes into commercially-acceptable vegetable corns is limited. This has prompted the present studies of (a) the establishment of double mutants involving the opaque-2 ($\underline{o}_2$) and floury-2 ($\underline{fl}_2$) genes in combination with brittle-1 ($\underline{bt}_1$), brittle-2 ($\underline{bt}_2$), shrunken-2 ($\underline{sh}_2$) and sugary-1 ($\underline{su}_1$) genes into a Hawaiian AA8 inbred, a tropical CM104 inbred and Hawaiian composite backgrounds, and (b) the determination of the biological values for single and double mutant genotypes.

REVIEW OF LITERATURE

Three mutations are known to greatly influence amino acid composition of maize endosperm protein. These mutations are opaque-2 (chromosome 7:16), floury-2 (chromosome 4:63) and opaque-7 (chromosome 10) (McWhirter, 1973). The o_2 mutant was isolated by Jones and Singleton and the fl_2 mutant by Mumms (Emerson et al., 1935). Both the o_2 and o_7 mutations are independently inherited as simple Mendelian recessives, while the floury-2 is semidominant. Opaque-2 is located on the seventh chromosome of maize (Singleton, 1939), and floury-2 was recently located on the fourth chromosome (Patterson, 1968).

Mertz et al. (1964) discovered that o_2 and floury-2 genes substantially increased the lysine content of maize endosperm. This was accompanied by a reduction in the zein to glutelin ratio. The Mertz group's discovery of a means of producing high-lysine corn (1964) generated widespread interest among plant breeders and nutritionists. It stirred the imaginations of many people who were concerned with the world-wide problem of protein need.

Nelson et al. (1965) reported that fl_2 had significantly higher methionine content than either normal or o_2, and fl_2 had nearly twice the tryptophane content present in normal endosperm. They reported that the protein content of the fl_2 genotype (13.6%) was higher than that of o_2 endosperms (11.1%), while Sprague et al. (1947) reported similar protein content (12%) for both mutants in different genetic backgrounds. The effect of the o_2 gene on amino acids persists in a high protein background (Mertz et al., 1965; Nelson, 1966). The gene does not affect

the amino acid composition of pollen and leaves (Nelson, 1969). Apparently the effects of both $\underline{o}_2$ and $\underline{fl}_2$ are limited to the endosperm tissue only (Mertz et al., 1966; Nelson, 1969) and there is little or no difference between amino acid composition of the protein in embryos taken from normal and $\underline{o}_2$ seeds.

Bates (1966) reported about a 35% increase in lysine of $+/\underline{o}_2/\underline{o}_2$ over normal endosperm but no apparent increase over normal was found in $+/+/\underline{o}_2$ endosperm. He also found dosage effect of $\underline{fl}_2$ for high-lysine content. Both Mossé (1966) and Mossé et al. (1966), working with entire seeds, and Jimenez (1966, 1968) working with protein fractionation of the endosperm, found that there is a substantial reduction in the amount of alcohol soluble fraction (the prolamines) synthesized in $\underline{o}_2$ and $\underline{fl}_2$ maize. A concomitant increase was observed in the relative proportions of the albumins, globulins and glutelins. Jimenez (1968) found that electrophoresced fractions from $\underline{o}_2$ endosperm had 3 bands less than the normal. Floury-2 also produced several proteins with altered electrophoretic mobilities.

The $\underline{o}_2$ gene has been associated with high ribonuclease activity in developing kernels (Dalby et al., 1967; Wilson and Alexander, 1967). Since the alcohol soluble zein proteins comprise more than 50% of total protein synthesized in normal maize endosperm, there are either numerous copies of the RNA messages for zeins present or the messages are more stable than those for other proteins. There is no evidence that allows a choice between these alternatives, but if the second were correct, the enhanced ribonuclease in the mutant would be expected to be more disruptive of zein synthesis than if the first pertained. In this view,

the synthesis of greater quantities of albumins and globulins in the mutant is then a secondary response following the partial repression of zein synthesis. Dalby and Cagampang (1970) in a further study of ribonuclease activity, found that $\underline{o}_2$ was effective in increasing ribonuclease activity until 16 days after pollination and concluded that the gene was inactive after that period. They also found a dosage effect of $\underline{o}_2$ for ribonuclease activity. No effect of $\underline{fl}_2$ gene on ribonuclease was observed. Cessation of $\underline{o}_2$ gene for ribonuclease activity at 15 days is unlikely (Nelson and Burr, 1973) in view of the completely recessive nature of the mutant allele, since the $\underline{o}_2$ phenotype is seen only in $+/\underline{o}_2/\underline{o}_2$ endosperms and the changes in amino acid profile are seen only in homozygous mutant endosperms (Bates, 1966).

Sodek and Wilson (1971) reported that when ^{14}C lysine was injected just below the developing ears, most of the label incorporated into protein in $\underline{o}_2$ was present in lysine, but in normal seeds much of the label was found in glutamate and proline. When labeled leucine was injected, most of the label recovered was in leucine for both genotypes. Goodsel (1968) found that normal and segregating kernels taken from the same ears contained significantly different quantities of potassium with the mean normal content being 0.37% and $\underline{o}_2$ 0.52%. Nelson (1969), Sodek and Wilson (1971) and Hansel et al. (1973) suggested that both $\underline{o}_2$ and $\underline{fl}_2$ are regulatory genes for zein synthesis.

Duvick (1961) showed that the site of zein deposition was the protein body. More recently, Wolf and his collaborators (1967) found that the protein bodies in $\underline{o}_2$ endosperm are about 1/20 of the diameter of protein bodies in normal maize. No protein bodies were detected in

fl_2 endosperm.

The o_2 gene has been associated with large embryo size (Watson, 1966; Witcher, 1966), lower grain yield and kernel weight, with loose packing of starch (Lambert et al., 1969; Sreeramulu and Bauman, 1970), high oil content, high percentage of cracked kernels (which make the kernels more susceptible to insects) and higher grain moisture at harvest (Lambert et al., 1969).

Studies of the wet milling properties of o_2 and normal maize indicated that the opaque-2 had an increased volume upon steeping and lower starch yield (Dimler, 1966; Watson, 1966; Watson and Yohl, 1967). The yield of usable grits on milling of o_2 and fl_2 was low and the flour by-product was high.

Other endosperm mutants have been associated with changes in protein and amino acid content in maize. Sprague et al. (1947) reported that the protein contents of bt_1, bt_2 and su_1 were 17%, 15% and 15% respectively. High protein values (about 17%) were also found by Teas and Teas (1953) in bt_2 kernels from segregating ears, while normal kernels from the same ears had 15% protein. The amount of tryptophane per kernel was found by Teas and Newton (1951) to be doubled in bt_2 as compared to the normal.

In studies of starch-modifying mutant genes and their combination with o_2, Glover et al. (unpublished data) found that the sugary-1 (su_1), shrunken-1 (sh_1), shrunken-2 (sh_2), shrunken-4 (sh_4), brittle-1 (bt_1) and brittle-2 (bt_2) genes increased the lysine content of the endosperm substantially above the isogenic normal control, and each gene had an enhanced effect on lysine when the gene was combined with o_2. Recently

Misra et al. (1972) separated the endosperm protein of $\underline{o}_2$, $\underline{o}_7$, $\underline{fl}_2$, $\underline{bt}_2$ and the double mutant $\underline{o}_2\underline{bt}_2$ into 4 soluble fractions by the Landy-Moureaux method. Compared to their isogenic normal counterparts, the mutant endosperms had high concentrations of albumin, globulins, and globulin-3, and lower concentrations of prolamines. The combination of $\underline{bt}_2$ and $\underline{o}_2$ had the greatest difference from the normal.

Several other genetic approaches to increasing protein quantity and quality have been tried. Tello et al. (1965) and Paez et al. (1969) found considerable natural variation in lysine content among field corn varieties and inbreds. Wolf et al. (1969) found high protein contents in kernels of amylose extender stocks, apparently due to the existence of multialeurone layers. This heritable characteristic seems to be independent of the amylose extender gene. Wolf et al. (1972) reported that Coroico, a South American race of floury maize, contained two to six layers of aleurone cells instead of the customary single aleurone cell layer found in ordinary dent corn. Total protein in aleurone of this variety is 35-38% compared with 22% in yellow dent corn. Lysine levels of the examined aleurone layers, both in Coroico and in yellow dent, ranged from 4.0 to 4.4% of total protein.

A phenomenon of irregular kernel arrangement (or pistillate inflorescence) on the cob of Country Gentleman ("shoe peg") sweet corn was reported by Stewart (1915). Huelsen and Gillis (1929) reported that the inheritance of irregular arrangement of corn kernels is digenic. The pistillate inflorescence develops secondary florets, which ordinarily abort during development. In shoe peg corns, these florets mature. The net result of the full expression of this phenotype is the

development of twice as many seeds as are normally produced on an ear of corn, thus increasing the total surface area of the protein rich aleurone layer. Nelson (1970) combined the pistillate inflorescence character with the opaque-2 gene, and was able to increase protein and lysine without decreasing yield.

Increased production of protein per hectare and increased percentage of protein in the seeds in response to the level of nitrogen fertilization was achieved (Anonymous, 1969). Nevertheless, these applications did not increase the protein quality; although the percentage of protein in the grain was increased, the percentage of tryptophane in the protein decreased.

Studies in rats (Mertz et al., 1965; Bressani et al., 1969), chicks (Cromwell, 1968), pigs (Pickett, 1966), cattle (Camp, 1972) and humans (Bressani, 1966; Bressani et al., 1969; Clark et al., 1967; Kies, 1970; Young et al., 1971 and Costance et al., 1972) have established the high protein quality of corn with the $\underline{o}_2$ gene compared to normal hybrid varieties. Nutritional studies with $\underline{fl}_2$ have indicated that it is superior to normal maize for the rat (Veron, 1967), the chick (Cromwell et al., 1968) and humans (Harpstead et al., 1969). The nutritive values of $\underline{o}_2$ have been attained with $\underline{o}_7$ in rats (Buttenshaw, 1973). Clark et al. (1967) found that eating 300g of the opaque-2 per day maintained either nitrogen equilibrium or positive balance of young adults. Bressani et al. (1969) reported that high-lysine $\underline{o}_2$ maize is similar to or higher (Young, 1971) than cow's milk in nutritional value and comparable to the protein of hen's egg. They concluded that if the quantity of protein in maize could be raised to 15% and made equal in quality to

that produced by $\underline{o}_2$ and $\underline{fl}_2$ genes, then children would grow normally on a diet of corn as the sole source of carbohydrates and protein, supplemented only with vitamins and minerals.

Glover et al. (1971) reported that $\underline{bt}_2/\underline{bt}_2/\underline{o}_2/\underline{o}_2$ with 17% protein and 4.4% lysine had a superior ratio of feed/gain of 2.2 compared to 2.7, 3.7, 5.1 and 7.4 for the $\underline{bt}_2$, $\underline{o}_2$, $\underline{fl}_2$ and normal genotypes respectively. They suggested that the $\underline{bt}_2\underline{o}_2$ double combination may be feasible in meeting the basic protein requirements for non-ruminant weanling individuals because of its very high quality protein and excellent biological feeding values.

RESULTS AND DISCUSSION

Six endosperm mutants were combined in the inbred CM104, AA8 and Hawaiian Sugar backgrounds to produce all possible high-lysine genotypes with opaque-2 and floury-2. The genes and their phenotypes and chromosomal location are summarized below:

Gene	Phenotype	Chromosomal location	Locus
su_1	sugary	4	71
bt_1	shrunken	5	22
bt_2	shrunken	4	near su_1 (71)
sh_2	shrunken	3	111.2
fl_2	opaque	4	63
o_2	opaque	7	16

1. Isolation of double mutant genotypes involving the opaque-2 gene

For the isolation of the double mutants, the order of presentation will be the following: opaque-2 sugary-1 (o_2su_1), opaque-2 shrunken-2 (o_2sh_2), opaque-2 brittle-1 (o_2bt_1) and opaque-2 brittle-2 (o_2bt_2). These double mutants will first be discussed in inbred CM104, followed by AA8 and finally in the Hawaiian Sugar (HS) background. The following abbreviations will be used throughout the text, I for (self), S for (sib) and TX for (test cross).

A sub-line of inbred CM104 (Brewbaker, 1971) carrying the o_2 gene was crossed with the respective CM104 sub-lines carrying su_1, bt_1, bt_2 and sh_2 genes. The F_1 plants were sib-pollinated and floury seeds (o_2o_2) were isolated from F_2 ears. Approximately 20 F_2 plants were self-pollinated, to produce ears segregating in an approximate ratio of

3 single mutants to 1 double mutant. The double mutants were back-crossed to o_2 CM104 and then selfed again to produce the double mutants.

Conversions of sugary-1 inbred AA8 (Brewbaker, 1971) had been completed to Su_1 and sh_2. These were crossed to o_2su_1 COMP 1 in order to produce double mutants with o_2. The (AA8 su_1 x bt_1 COMP 2) F_1 plants were crossed to AA8 (su_1) and their progeny crossed to o_2su_1 COMP 1d. Opaque, non-sugary kernels were planted, sibbed, and o_2 kernels again planted for self-pollinations producing the double mutant type. The o_2su_1 conversion is 75% isogenic to the AA8 line. The other 3 double mutants, (o_2sh_2), (o_2bt_1) and (o_2bt_2) of CM104 were isolated in similar fashion as o_2su_1. Summary of the isolation procedures in CM104, AA8 and HS are presented in Table 24.

From the 12 genotypes outlined in Table 24, 2 have been selected out for detailed description due to their outstanding merits; these are o_2su_1 and o_2sh_2 from the HS background. Seedlings of o_2sh_2 are vigorous, with erect stocks and are free from diseases mosaic, rust and blight. Plants produce long ears with huge kernels. Seedlings of o_2su_1 are not as erect as o_2sh_2, however they do have resistance to the three diseases.

These two double mutants were obtained in the following way: (o_2su_1 COMP 1c x HSsu_1) x (sh_2 Syn 2 x sh_2 COMP 3)S,I,S. The F_1 plants from (o_2su_1 COMP 1c x HSsu_1) were homozygous for the sugary-1 gene, and heterozygous for the o_2 locus. These were crossed to F_1 plants from (sh_2 Syn 2 x sh_2 COMP 3). Seeds from the double cross were planted, and a large number of plants that were free from rust, mosaic and blight were sib-pollinated. Only opaque seeds from the sibs were planted (in

Table 24. Crossing procedures used to obtain the double mutant genotypes involving the opaque-2 gene in 3 backgrounds.

Backgrounds	Genotypes	Crosses
CM104	$\underline{o_2su_1}$	(CM104$\underline{o_2}$ x CM104$\underline{su_1}$)S,I)CM104$\underline{o_2}$)I
	$\underline{o_2sh_2}$	(CM104$\underline{o_2}$ x CM104$\underline{sh_2}$)S,I)CM104$\underline{o_2}$)I
	$\underline{o_2bt_1}$	(CM104$\underline{o_2}$ x CM104$\underline{bt_1}$)S,I)CM104$\underline{o_2}$)I
	$\underline{o_2bt_2}$	(CM104$\underline{o_2}$ x CM104$\underline{bt_2}$)S,I)CM104$\underline{o_2}$)I
AA8	$\underline{o_2su_1}$	($\underline{bt_1}$* COMP 2 x AA8)AA8$\underline{su_1}$ x $\underline{o_2su_1}$ COMP 1d)S_3
	$\underline{o_2sh_2}$	($\underline{o_2su_1}$ COMP 1d x AA8$\underline{sh_2}$)I_2
	$\underline{o_2bt_1}$	($\underline{bt_1}$ COMP 2c x AA8)AA8$\underline{su_1}$ x $\underline{o_2su_1}$ COMP 1d)S)AA8$\underline{bt_1}$)S,I
	$\underline{o_2bt_2}$	($\underline{o_2su_1}$ COMP 1d x $\underline{bt_2}$ COMP 1)AA8$\underline{Su_1}$)S,I,S_2
HS	$\underline{o_2su_1}$	($\underline{o_2su_1}$ COMP 1c x HS$\underline{su_1}$)$\underline{sh_2}$* Syn 2 x $\underline{sh_2}$* COMP 3)S,I,S
	$\underline{o_2sh_2}$	($\underline{o_2su_1}$ COMP 1c x HS$\underline{su_1}$)$\underline{sh_2}$ Syn 2 x $\underline{sh_2}$ COMP 3)S,I,S,
	$\underline{o_2bt_1}$	($\underline{o_2su_1}$ COMP 1c x $\underline{bt_1}$ COMP 2)$\underline{bt_1}$ COMP 2e)S,I
	$\underline{o_2bt_2}$	($\underline{o_2su_1}$ COMP 1c x $\underline{bt_2}$ COMP 1)S,I

*Crosses were made for many purposes besides conversions and the appropriate types were selected out.

3 long rows, approximately 600 hills). Selection against mosaic, rust and blight was practiced as in the previous generation, and all remaining healthy plants were selfed. Some ears were completely homozygous for $\underline{o}_2$, some were segregating for $\underline{su}_1$ or $\underline{sh}_2$ and still others were segregating for both $\underline{sh}_2$ and $\underline{su}_1$ on the same ear. Both the $\underline{su}_1$ and $\underline{sh}_2$ kernels segregated on the $\underline{o}_2$ background in an approximate ratio of 3 opaque to 1 double mutant. Segregation of $\underline{su}_1$ and $\underline{sh}_2$ on the same ear, produced a ratio of 9 opaque to 3 sugary to 3 shrunken to 1 sugary-shrunken ($\underline{o}_2\underline{su}_1\underline{sh}_2$) triple mutant.

Ears segregating for either sugary or shrunken kernels were isolated. A total of 7 long rows were planted in the winter of 1973; three for $\underline{o}_2\underline{su}_1$ COMP 3, two for $\underline{o}_2\underline{sh}_2$ COMP 1, and 2 rows for the sugary-shrunken mixture (probably still heterozygous for either the $\underline{su}_1$ or $\underline{sh}_2$ gene). Continued intensive selection against the 3 diseases was practiced. The $\underline{o}_2\underline{su}_1$ and $\underline{o}_2\underline{sh}_2$ were to have been sibbed and the mixture (sugary-shrunken) selfed, however, due to heavy winter rainfall and storms at the time of pollination, all plants were lost. Fortunately about 300 plants, each of $\underline{o}_2\underline{su}_1$ and $\underline{o}_2\underline{sh}_2$, had been planted one month earlier and all were recovered. The sibbed ears of $\underline{o}_2\underline{sh}_2$ were short but the kernel size was extremely large. Seeds of $\underline{o}_2\underline{sh}_2$ were again planted in the spring of 1974. Seedlings were vigorous with erect stalks and approximately 80% of the plants were free from mosaic, rust and blight. Plants were sibbed and produced very long ears with large kernels.

In all three backgrounds, the genes $\underline{su}_1$, $\underline{sh}_2$, $\underline{bt}_1$ and $\underline{bt}_2$ were epistatic to $\underline{o}_2$ in that the double mutants $\underline{o}_2\underline{su}_1$, $\underline{o}_2\underline{sh}_2$, $\underline{o}_2\underline{bt}_1$ and $\underline{o}_2\underline{bt}_2$ were all wrinkled (sugary) or shrunken. For this reason the

classification for the segregation of these genes in the o_2 background was best.

2. Isolation of double mutants involving the floury-2 gene

Floury-2 behaves as a completely dominant gene in the CM104 background, whereas its inheritance is semi-dominant in less flinty backgrounds. Because of this mode of inheritance, fl_2 requires two generations to recover homozygous floury-2 lines from heterozygous plants rather than just one as with the opaque-2 gene.

The establishment of the double mutants will be discussed in inbred CM104 in the following order of occurrence: fl_2bt_1, fl_2sh_2, fl_2su_1 and fl_2bt_2 (Table 25).

The fl_2bt_1 genotype was established in the following way: F_1 plants from (CM104 x bt_1 COMP 2) were sibbed and the bt_1 seeds were backcrossed to CM104fl_2. Floury seeds were planted, selfed and the shrunken kernels were isolated. The putative double mutant (fl_2bt_1) kernels were selfed and crossed to a normal tester (++). The test crossed ears were either completely floury (fl+) or they were segregating in a 1 flinty (++) to 1 floury (fl+). The only ears saved were those that were confirmed homozygous for the fl_2 gene. The fl_2sh_2 genotype was obtained by crossing (CM104fl_2 x sh_2 COMP 2). The F_1 seeds were sib-pollinated and the plants from sh_2 kernels were selfed and test crossed to a tester as in fl_2bt_1.

The isolation of the double mutant genotypes fl_2su_1 and fl_2bt_2 was expected to present some problems since the su_1 and bt_2 genes are tightly linked to fl_2. The fl_2su_1 genotype was isolated by crossing

Table 25. Crossing procedures used for the isolation of the double mutants involving the floury-2 gene.

Background	Genotype	Crosses
CM104	$\underline{fl}_2\underline{bt}_1$	(CM104$\underline{fl}_2$ x $\underline{bt}_1$ COMP 2)S)CM104$\underline{fl}_2$)I_2TX
	$\underline{fl}_2\underline{sh}_2$	(CM104$\underline{fl}_2$ x $\underline{sh}_2$ COMP 2)S,I,TX
	$\underline{fl}_2\underline{su}_1$	(CM104$\underline{su}_1$ x CM104$\underline{fl}_2$)S,I,TX)I
	$\underline{fl}_2\underline{bt}_2$	(CM104$\underline{fl}_2$ x $\underline{bt}_2$ COMP 1)CM104$\underline{fl}_2$)CM104$\underline{bt}_2$)I,TX)I

CM104su_1 x CM104fl_2. The F_1 plants (+su_1;fl_2+) from the original cross (su_1+;su_1+ x +fl_2;+fl_2) were sib-pollinated. Only sugary kernels from the sibs were isolated, (fl_2su_1;+su_1, +su_1;+su_1), and then self-pollinated. Plants from sugary seeds were also crossed to a normal female tester (++). Since kernels resulting from this test cross were translucent in phenotype, ++;su_1su_1 was assigned to tested parents.

Only a few ears of the test cross were segregating for approximately 1 translucent (++) to 1 floury kernel (++;su_1fl_2). The double mutants were derived by further selfing the opaque kernels (++;fl_2su_1) of the test cross which then segregated in a 1 flint (++) to 1 sugary (su_1su_1;fl_2fl_2) ratio. Wrinkled translucent kernels were selected.

The homozygosity of the fl_2 gene in the isolated double mutants was further confirmed by selfing the first ear and test crossing the second ear (of the same plant) with (++) pollen, or by splitting the silk (of a previously covered ear) and selfing the one side and test crossing the other side of the silk with pollen from a (++) tester.

The isolation of fl_2bt_2 followed exactly the same procedure as described for fl_2su_1, except that the number of plants recovered from the putative double mutants were less because of poor germination, but with a higher frequency of crossing over than between su_1 and fl_2 genes. Although there was a tendency for endosperms of fl_2bt_2 to be slightly opaque, classification was only possible where the bt_2 gene was secregating in a fl_2fl_2 background.

The genes su_1, bt_1, bt_2 and sh_2 were epistatic to fl_2 in that the double mutants, fl_2su_1, fl_2bt_1, fl_2bt_2 and fl_2sh_2 were wrinkled or shrunken. For this reason the classification for the segregation of

these genes in the $\underline{fl}_2$ background was best.

3. Background effect of the double mutants

The double mutants ($\underline{o}_2\underline{su}_1$, $\underline{o}_2\underline{sh}_2$, $\underline{o}_2\underline{bt}_1$, $\underline{o}_2\underline{bt}_2$ and $\underline{fl}_2\underline{su}_1$, $\underline{fl}_2\underline{sh}_2$, $\underline{fl}_2\underline{bt}_1$, $\underline{fl}_2\underline{bt}_2$) were isolated from CM104, AA8 and HS backgrounds. CM104 and AA8 are two tropical inbreds, HS is a variety and is highly variable. The purpose of this study, therefore, was to observe the behavior of these 8 mutants in each of the 3 backgrounds. The double mutants involving the $\underline{sh}_2$, $\underline{bt}_1$, and $\underline{bt}_2$ genes, in combination with either the $\underline{fl}_2$ or $\underline{o}_2$ genes, were poor in germination in both CM104 and AA8 backgrounds. Most of the double mutants isolated from the HS background were better in germination than those established from the other two inbreds. Further studies of the 12 genetic stocks are in progress.

ANIMAL FEEDING STUDIES

MATERIALS AND METHODS

Experiment 1

Six genetic stocks were used in the following study of rat and vole nutrition (Table 26). Opaque-2 corn (Diet E) was a commercial sample provided by Albers Milling Co., and the field corn (Diet F) was hybrid H609, a modified B37 x B14 corn available from Dr. Brewbaker. Opaque-2 sweet corn (Diet C) was derived from Hawaiian composite $\underline{o_2}$ $\underline{su}$ COMP 1d. Sweet corn ($\underline{su_1}$) (Diet D) was the H68 hybrid. Floury-2 shrunken-2 ($\underline{fl_2 sh_2}$) (Diet A) supersweet corn was derived from a Hawaiian $\underline{fl_2 sh_2}$ COMP 1b. Shrunken-2 ($\underline{sh_2}$) corn was derived from Hawaiian $\underline{sh_2}$ Syn 2g.

The H68, $\underline{o_2 su_1}$, $\underline{sh_2}$ and $\underline{fl_2 sh_2}$ corns were harvested 18 days after pollination (DAP). Both sweet and supersweet corns were lyophilized. The 6 diet samples were ground in a Wiley mill to pass a 0.0331 inch screen. Protein concentrations were determined by a modified Biurtet procedure (Parial, 1970). The 6 diets, their genotype, description and protein percent are summarized in Table .

Biological assays were conducted on weanling female rats (local Wistar strain) fed the 6 different samples of corn for an 18 day period. Five weanling rats, weighing between 32 and 48 grams, were randomly allocated to each group. They were individually housed in wire mesh cages and given unrestricted access to one of 6 diets (Table 26). All of the 6 diets were composed of 90 percent corn, 5 percent corn oil, 4 percent salt mixture and 1 percent vitamin fortification mixture

Table 26. Six diets with their genotypes, descriptions and protein percents.

Diets	Genotype	Description	Protein %
A	$\underline{fl_2fl_2};\underline{sh_2sh_2}$	high-lysine, high-sucrose corn	13.90
B	$\underline{sh_2sh_2}$	high-sucrose corn	13.40
C	$\underline{su_1su_1};\underline{o_2o_2}$	high-lysine sweet corn	12.88
D	$\underline{su_1su_1}$	sweet corn	11.46
E	$\underline{o_2o_2}$	high-lysine field corn	10.95
F	$\underline{+\ +}$	field corn	12.37

(Mertz, 1966). The rats were weighed and food consumption was determined at 4 day intervals. Spillage losses from individual rat cages were determined daily.

Experiment 2

This study compared the nutritional value of 3 kinds of corn, as a sole diet with a complete commercial diet for quails. Four diets were used, two from the previous experiment (diets C and D) and two new diets, G (field corn, 8.7% protein) and H (a commercial complete diet, 21.0% protein).

Biological assays were conducted on unsexed Japanese quails (Quaternex) and were fed 3 corn diets for two weeks. A complete commercial diet was included for 7 days during the second week of the experiment. Twelve quails weighing between 19 and 35 grams were randomly assigned to the 3 diets. The twelve quails assigned to the commercial diet, during the second week, weighed between 29 and 49 grams. The birds were given wing band numbers for identification, and placed in electrically heated battery brooders with raised wire floors. Feed and water were provided ad libitum. Quails and feed were weighed at weekly intervals until the termination of the experiment. Diets C ($\underline{o}_2\underline{o}_2\underline{su}_1\underline{su}_1$), D ($\underline{su}_1\underline{su}_1$) and G ($\underline{++}$) were 100% corn. Diet H (commercial diet for quails) was a balanced diet.

RESULTS AND DISCUSSION

Experiment 1

The rat feeding experiment consisted of a completely randomized design with 5 samples and 6 types of corn as treatments (described in Table 26). Analysis of variance was conducted on data up to the 18th day of feeding. The average food intakes, gains in weight, feed/gain (F/G) ratios, and protein efficiency ratios (P.E.R.) are presented in Table 27. Slopes of the regressions (b) of feed consumption of body weight, in grams, are also given.

Highly significant differences were observed among the 6 dietary treatments (Table 28). The average weight gain varied from 27.6 gm. for diet F (161.62 gm. feed intake) to 65.88 gm. for diet A (191.25 gm. feed consumption). Based on weight gains, diet A (shrunken-2 floury-2) out-ranked all other diets in nutritional values followed by diet B, C and E, D and F. The gains in weight, as a function of days (or times) is illustrated in Figure 10. Growth was essentially linear during the period of study.

Treatment A had the lowest feed/gain ratio (F/G) in comparison to all other treatments. The F/G ratio varied from 2.63 for diet A to 5.93 for diet F. Rats fed supersweet diets A and B had about the same F/G ratio and were highly significantly lower than all other diets. The F/G ratio from rats fed diet F were highly significantly greater than that of diet D.

The nutritional value of the 6 diets can be viewed in terms of slopes of regression of feed consumption on body weight (Figure 11).

Table 27. Average food intake, gain in weight, feed/gain, b and protein efficiency ratio (P.E.R.), for rats and voles. Rats were fed 6 diets for a duration of 18 days.

Diets	Genes	Avg. food intake (gm.)	Avg. Gain	Feed/Gain Ratio	b	P.E.R. rat	P.E.R. vole
A	$\underline{sh}_2\underline{fl}_2$	191.25	65.88 a	2.63 a	2.66	2.72 a	2.52 a
B	$\underline{sh}_2$	183.50	55.88 b	2.82 a	3.07	2.48 b	2.06 ab
C	$\underline{su}_1\underline{o}_2$	225.00	42.80 c	4.09 b	3.93	1.85 d	1.72 ab
D	$\underline{su}_1$	190.00	29.60 d	4.84 c	4.58	2.08 c	1.50 ab
E	$\underline{o}_2$	234.50	47.80 c	4.17 b	4.16	2.18 c	1.63 ab
F	-	161.62	27.60 d	5.93 d	5.74	1.50 e	1.22 c

Table 28. Analyses of variance for average weight, Feed/Gain (F/G) and protein efficiency ratio (P.E.R.) for rats and voles from Table 27.

Source	df	Gain wt. in grams	F/G	P.E.R. rat	P.E.R. vole
Treatment	5	1,170.22**	7.36*	0.96*	1.03*
Error	24	22.58	0.16	0.02	0.15
DLSD @ 0.01		6.84	0.58	0.17	0.59
CV		11%	10%	6%	22%

Figure 10. Average gains of rats fed 6 diets of corn (Table 27).

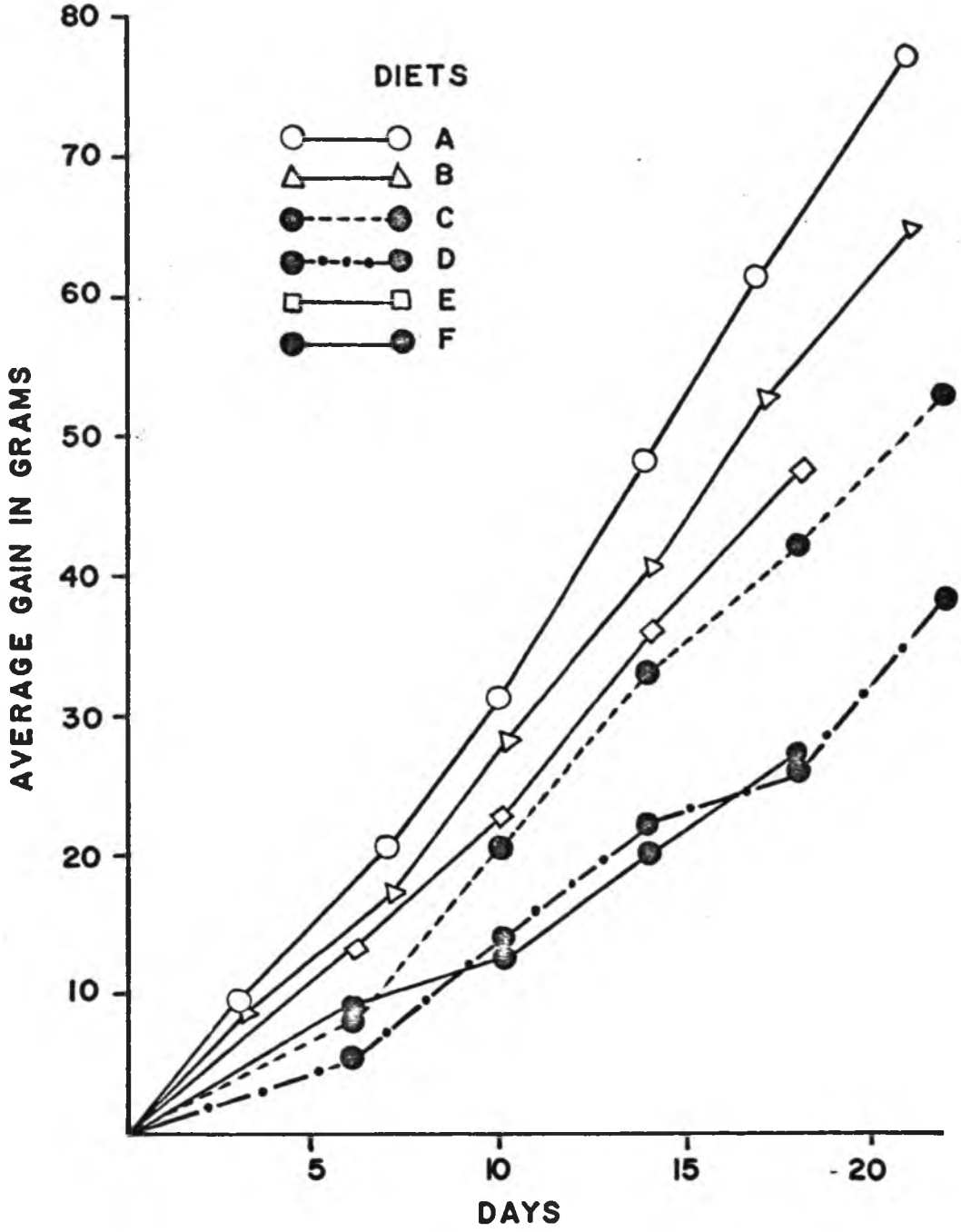
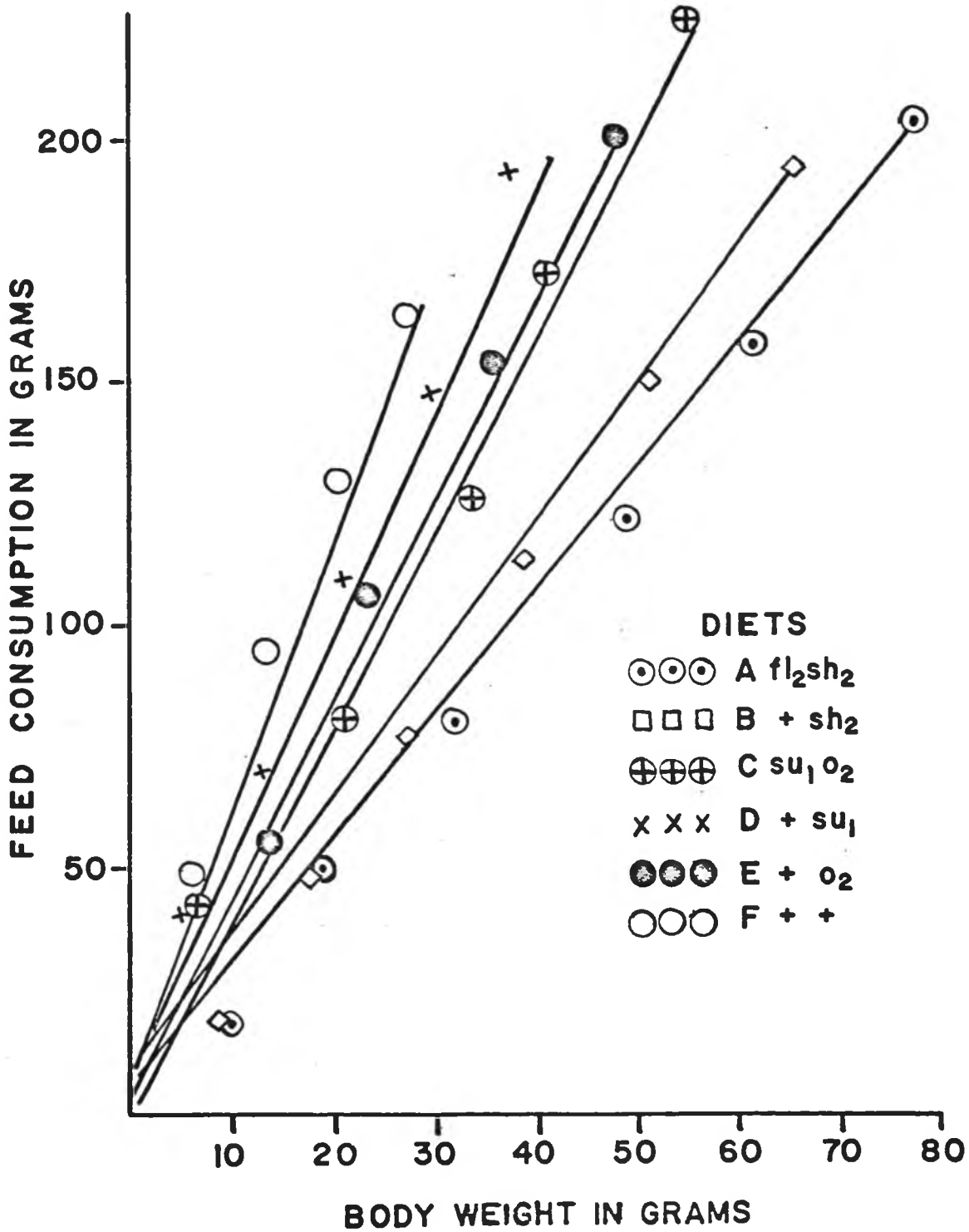

DIETS
A
B
C
D
E
F
AVERAGE GAIN IN GRAMS
80
70
60
50
40
30
20
10
5
10
15
20
DAYS

Figure 11. Regression of feed consumption (y) on body weight (x) for 6 diets fed to 5 rats (Table 27).


FEED CONSUMPTION IN GRAMS
200
150
100
50
10
20
30
40
50
60
70
80
BODY WEIGHT IN GRAMS
DIETS
A fl_2sh_2
B + sh_2
C su_1o_2
D + su_1
E + o_2
F + +

The lower the b value, the better is the quality of the diet; that is, more gain in weight with less feed consumption. The lowest value for b was found in diet A followed by B and C then D and E. Diet F had the highest value in b. It is interesting to note that although rats fed diet E (high-lysine field corn) had a higher average food intake than rats fed diet A (high-lysine supersweet corn), and diet E had gains that were relatively low compared to diet A.

An additional statistic calculated for evaluation of biological value is the protein efficiency ratio (P.E.R.). The P.E.R. ranged from 1.50 for diet E to 2.72 for diet F. The biological values of the 6 diets, based on P.E.R., are the following: diet A was highly significantly better than diet B; diet B was highly significantly better than both diets D and E. Unexpectedly, diet D (sweet corn high-lysine) was evaluated as lower in nutritional value than either diets C (a straight sweet corn H68) or diet E (high-lysine field corn). The nutritional quality of field corn (diet F) was extremely inferior to the rest of the diets.

In all 3 paired diets, ($\underline{sh}_2$ vs $\underline{fl}_2\underline{sh}_2$, $\underline{su}_1$ vs $\underline{o}_2\underline{su}_1$, $\underline{o}_2$ vs $\underline{+}\underline{+}$) the biological feeding values of high-lysine corn diets (either with $\underline{o}_2$ or $\underline{fl}_2$ genes) as revealed in average gains F/G ratios, b's and P.E.R. ratios, were highly superior to their counterpart diets. Diet C (sweet corn high-lysine) was highly significantly better than diet D in all categories, except for the P.E.R. ratio. Diet E had better nutritional values than diet F in all categories measured.

The superiority of high quality protein in $\underline{fl}_2\underline{fl}_2$ $\underline{sh}_2\underline{sh}_2$ over $\underline{sh}_2\underline{sh}_2$ genotype is unequivocally demonstrated in this study. Protein

quality in the $\underline{sh}_2$ genotype appeared to be better than the protein of $\underline{su}_1$, $\underline{o}_2$ or the double mutant genotype $\underline{su}_1\underline{o}_2$. Quality of protein from sweet corn ($\underline{su}_1$) was as good as that of high-lysine field corn ($\underline{o}_2$), and both were highly significantly better than field corn. Protein quality of the double mutant $\underline{o}_2\underline{su}_1$ was highly significantly better in nutritional value than protein derived from each genotype alone and it was superior on the basis of F/G and slope (b), however it was lower in P.E.R. than diets C and E.

In all of the 3 paired tests, feed was not prepared on an isonitrogenous or isocaloric basis (protein concentrations are in Table 26). It is preferable that such tests are conducted at low protein levels (Mertz, 1966) because animals are extremely sensitive to the quality of protein fed. The fact that protein levels were at optimum levels for rats in diets C (12.88%) and D (11.46%) may account for the lack of discrimination in P.E.R. values of the two diets.

The same six diets were sent to Dr. F. C. Elliott at the Department of Agronomy, University of Michigan for the determination of P.E.R. using voles. Five voles were fed the 6 diets on an isonitrogenous basis of 7.2% protein. The data from Dr. Elliott are reported in Table 28. In the process of handling or packaging the samples, diets E and F appear to have been switched, and were restored to their confirmed order. Highly significant differences between the 6 diets are shown (Table 28). Only diet F was highly significantly different from the rest of the diets.

Lack of discrimination in nutritional value of the 6 diets by vole can be attributed to these factors: small quantities of diet

(approximately 250 gm. was consumed by the voles compared to 1500 gm. consumed by the rats), also a short duration of the experiment (5 days for the voles compared to 18 days for the rats), small initial body weight of the vole (2 to 3 gm. vs 40 to 50 in the rat) and larger coefficient of variations for voles (22%) than for rats (6% C.V.).

Experiment 2

The results of weight gain or loss by quails fed 4 different diets is summarized in Table 29. Two quails on diet C died the first week, several lost weight, and the latter either maintained their initial weight or gained slightly. An additional quail was lost the following week.

A much more severe loss was recorded for birds fed diet D (sweet corn, $\underline{su_1}\,\underline{su_1}$). During the first week 5 quails died and the remaining suffered serious losses in weight. Two more quails died the following week, leaving 5 birds in the experiment.

Quails fed diet G (field corn) experienced similar loss in weight as patterned in diets C and D. It was surprising, though, that for the duration of the experiment (14 days) no mortality occurred.

Gain in weight by a group of 12 quails fed diet H (a commercial complete diet for quails) was extremely high. The differential responses by quails fed these 4 diets are shown graphically in Figure 12.

Diet D (sweet corn $\underline{su_1}\,\underline{su_1}$ $\underline{++}$) presented some physical problems during feeding for the birds. Due to the sticky nature of the feed, the bird's beaks were glued together. Conditions were heightened by the presence of an open pan of water which was available to the group of 12

Figure 12. Weekly gain in weight by quails fed 4 diets.

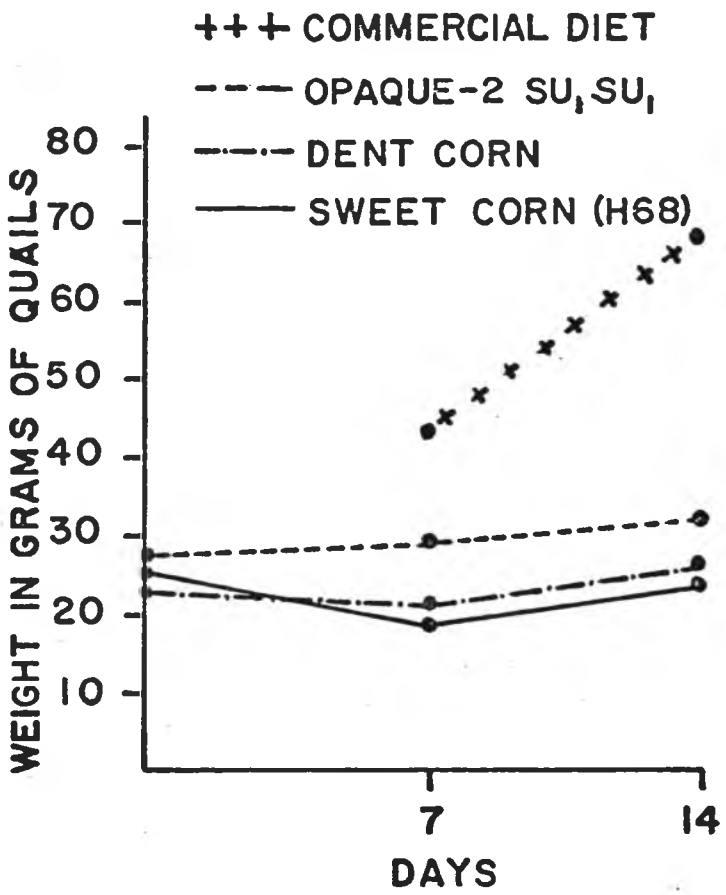

+++ COMMERCIAL DIET
--- OPAQUE-2 SU, SU,
-·-·- DENT CORN
—— SWEET CORN (H68)
WEIGHT IN GRAMS OF QUAILS
80
70
60
50
40
30
20
10
7
14
DAYS

Table 29. Initial weight of 36 quails and weight at first and second week after feeding four diets C, D, G and H.

Diets	C			D			E			H	
Quail Wing Band	o_2su_1 COMP 1e high-lysine sweet corn initl. wt.	1	2	H68 sweet corn initl. wt.	1	2	field corn initl. wt.	1	2	Commercial feed 1	2
1	35	35	37	24	-	-	21	16	20	46	68
2	25	24	27	25	22	22	21	18	21	49	74
3	29	32	32	28	-	-	25	23	26	47	73
4	24	23	24	25	22	-	24	20	26	44	71
5	26	25	27	20	-	-	22	20	24	45	68
6	29	-	-	29	23	28	24	20	24	40	62
7	26	-	-	23	-	-	21	29	34	44	70
8	31	36	37	24	22	23	20	15	23	48	71
9	26	27	-	24	19	21	19	15	18	29	53
10	20	20	23	23	-	-	33	32	40	46	73
11	32	35	37	28	20	22	19	19	22	49	70
12	31	31	37	28	10	-	26	23	27	36	61
Average	27.0	28.8	27.1	30.1	19.7	23.2	22.9	20.8	25.4	43.6	67.8

quails.

Quails were designated unsexed because phenotypic sex differentiation does not occur until the 4th or 5th week after birth. Males have better response in body weight than do females, thus, lack of sex differentiation and high methionine requirement during the feather development in this experiment confounds the nutritional value of the diets.

It is quite obvious that the three diets C, D and G were far below the protein requirement for quails and that they were lacking in minerals and vitamins.

Low quantities of feed, low protein levels, low methionine requirement, easy sex differentiation and short duration of the experiments for rats makes this species much more desirable for nutritional bioassay of corn than either quails or voles.

CHAPTER FOUR

PERICARP SELECTION

INTRODUCTION

Pericarp is the seed coat of cereals, a diploid maternal tissue developing independently of the embryo and endosperm. Consequently on a weight basis, reduced endosperm content increases the pericarp percentage. This is more pronounced in the high-sucrose corns, where the amount of endosperm is drastically reduced. In mature dent corn (Zea mays L.) the kernel consists of 5 to 6% pericarp tissue (Wolf et al., 1952). In endosperm mutants such as ae, the pericarp makes up to 10% of the kernel weight owing to reduced endosperm development (Zuber at al., 1960).

Almost 50% of the pericarp is hemicellulose (Wolf et al., 1952) which is highly undigestable. A high percent of pericarp in sweet and supersweet corn essentially decreases the nutritional value of these corns. Since consumers want a raw material that will give them a maximum yield of prime product, kernels with the least pericarp tissue should mean more recovery of the most digestable material. The total amount of pericarp tissue could be lowered by reducing its thickness, assuming that thickness and weight are positively correlated. Helm and Zuber (1972) estimated heritability in the narrow sense of dent pericarp thickness to be 80%. They concluded that breeding procedures that would take advantage of large proportions of line effects should be successful in selection for either thin or thick pericarp.

The objectives of this study were two fold: to select for thin and thick pericarp in sweet corn and to study the development of pericarp thickness and protein synthesis of sweet and high sucrose corn.

REVIEW OF LITERATURE

The quality of sweet corn is determined primarily by the degree of succulence, the quantity and toughness of the pericarp and the flavor. Culpepper and Magoon (1924) reported toughness of the pericarp to be an important quality factor in sweet corn. The nutritive value of pericarp tissue is lower than the remainder of the kernel (Hopkins, 1903).

Bailey and Bailey (1938) reported that sweet corn varieties with low puncture indexes generally have thin pericarp. As the kernel matures, the pericarp decreases in thickness and resistance to mechanical puncture markedly increases.

Haddad (1931) studied the relationship between inbred lines and their F_1 hybrids. He found the pericarp to be thicker on the side of the kernel than over the crown. The differences in thickness were due to reduced cell wall thickness and not to changes in cell numbers.

Wolf et al. (1952) made an extensive study of the structure of the mature corn kernel and found rather constant cell numbers in dent corn pericarp, ranging from 17 to 22 on the germinal face and from 19 to 25 cell layers on the abgerminal side, again indicating that differential thickness isa function of cell wall thickness.

Several devices have been designed to measure the toughness of sweet corn pericarp (Huelsen, 1954; Twig and Kramer, 1956; and Kramer, 1952). Some of these measure the force required for penetration of the pericarp by a needle of given size (Huelsen, 1954). Others measure the moisture by vacuum oven (AOAC, 1945), Stainlite (Anonymous, 1950), and Brow-Duvel (Scott, 1939) and succulence by a succulometer (Kramer et al., 1946) and Shear press (Kramer et al., 1951). Still others split the kernels in

half with a sharp knife, dip the pieces in iodine solution to stain the endosperm and measure the unstained pericarp with an ocular micrometer (Eden, 1953). Microscopic measurements of pericarp thickness and cell counts in fresh frozen section was carried out by Wolf et al. (1952) who then determined the percentage of dry weight of the pericarp in relation to total dry weight of whole kernels (Wolf et al., 1969).

A somewhat different test designed to measure the proportion of pericarp in sweet corn has been devised (Kramer et al., 1949; McArdle and Dersosier, 1954). Kernels blended in a Waring blender, are filtered through a screen and the shredded pericarp computed. None of these methods have proved to be completely successful, partly due to inaccuracies in the measuring devices.

An inexpensive, dependable method is the micrometer method reported by Wolf et al. (1969). They also reported that genotypes which have pericarp thickness differences of 20 microns should be easily separated by this method unless they involve the recessive sugary ($\underline{su}_1$) alleles. Helm and Zuber (1969) reported pericarp thickness ranging from 62 to 160 microns among 3 corn inbred lines.

Richardson (1960) reported on the inheritance of pericarp thickness in popcorn. He concluded that a single major gene is responsible for thin pericarp, and that a modifier gene complex conditions the production of thick pericarp.

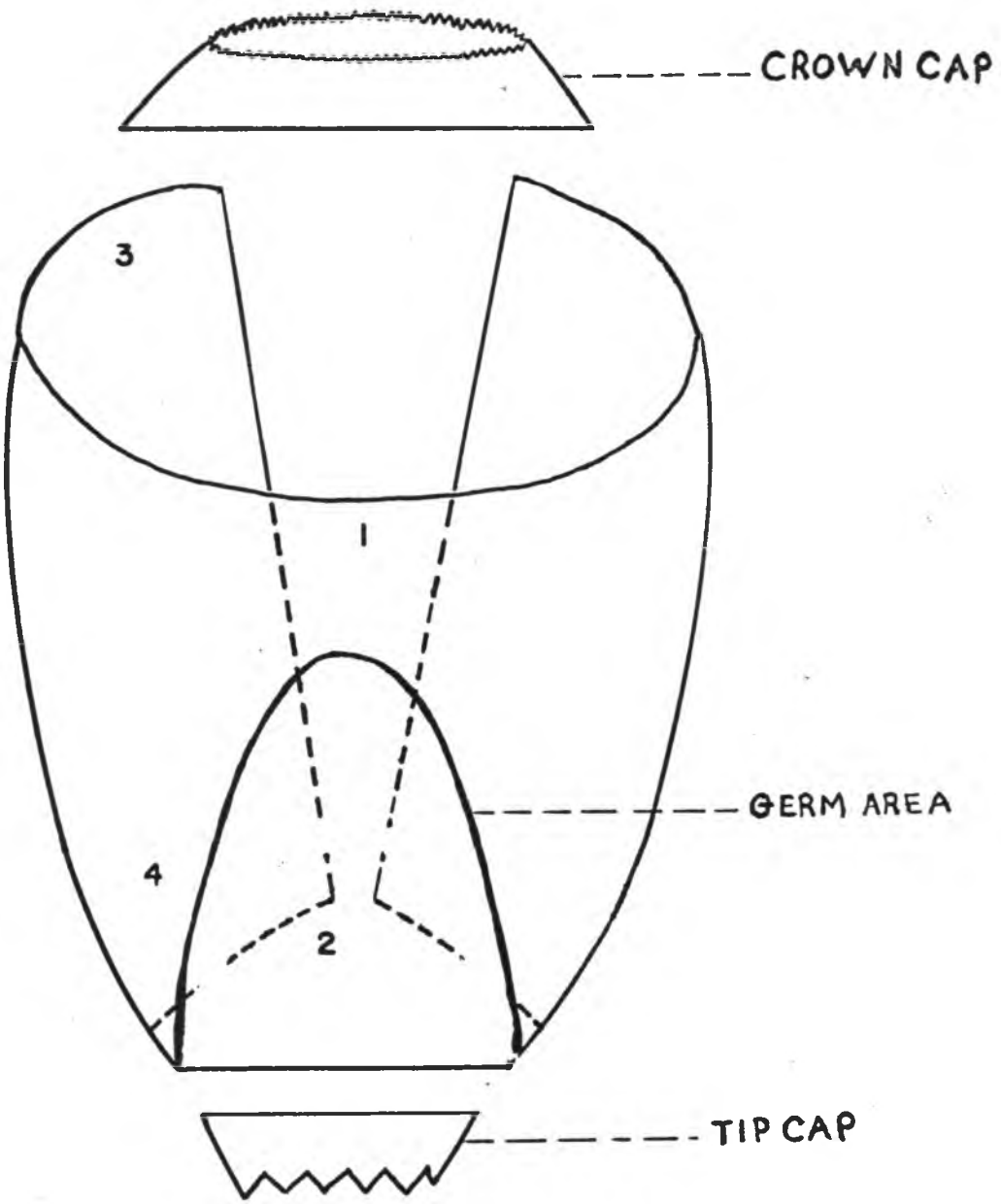
Helm and Zuber (1970) studied the pericarp thickness of six F_1 and F_2 hybrids from all possible combination crosses among four white dent corn inbred lines (H30, Molw, 33-16 and K41). The mean pericarp thickness of all F_2 hybrids exceeded F_1 hybrids by about 2 microns.

MATERIALS AND METHODS

Pericarp measurement

All pericarp thickness measurements were obtained by the following procedure: each freshly harvested ear was sampled in a uniform manner. Ears were frozen and 7 duplicate kernels were removed from three kernel rows midway in the lower half of the ear. Kernels were refrozen, enabling them to become hard and solid for easy cutting and removal of the pericarp. The crown and tip cup portion of each kernel were removed with a sharp razor blade. The pericarp was slit along the abgerminal side of the kernel and the pericarp was then removed. The resulting shape of the pericarp was a rectangular strip with both flat surfaces, germinal and abgerminal, away from the cut edge. Excised pericarps were cleaned with facial tissue and placed in 1:3 (v:v) water glycerol solution and evacuated in a vacuum desiccator. After evacuation they were allowed to stand for 24 hours. Pericarp strips were then blotted dry with facial tissue and placed in petri dishes and allowed to equilibrate for 24 hours. The equilibration environment was approximately 20°C and 55% relative humidity. Measurements were taken on each strip, as shown in Figure 13, with an Ames model #56216 micrometer. Thickness readings were recorded in microns. Four positions were measured as shown in Figure 13.

Figure 13. Diagram of pericarp and tip cap indicating peeling procedure and sites 1-4 of measurements. One and two are the germinal side, three and four are the abgerminal side.


CROWN CAP
3
1
GERM AREA
4
2
TIP CAP

RESULTS AND DISCUSSION

1. Selection for pericarp thickness in opaque-2 sugary-1 COMP 2

Selection experiments for thin and thick pericarp in $o_2 su_1$ COMP 2 (Brewbaker, 1971) were initiated in 1973. Seeds from the original population were planted and prior to anthesis, 500 plants with erect stalks (from approximately 4,000 plants) were selected. The ears from the selected plants were covered with glassine shoot bags. At anthesis, when all shoots had developed a sizable portion of their silk, glassine bags were removed and open-pollination took place. Selected ears were painted in red and this was considered as zero days after pollination (DAP).

At 22 (DAP) 50 ears were harvested and bite-tested. They were divided into 2 groups, tender and tough. These ears were used for further selection experiments in the two directions.

Seeds of cycle 1, selected for tough pericarp, were planted ear to row. Three short rows were planted of each ear, with an alternating row of seeds bulked from the selected 25 ears, this row to be used as a male parent. At anthesis ears were hand sib-pollinated and 32 of the toughest ears were selected on the basis of bite tests at 23 DAP. Selection for tenderness was carried out in similar fashion, the only notable difference was that in germination, kernels selected for tenderness were lower in viability than the tough kernels.

Pericarp thickness was measured by a micrometer, (Ames #56216). Thickness was measured for tender ears only. Kernels were divided into 2 groups, thin pericarp (52 to 66 microns thick) and thick pericarp

(86 to 108 microns thick). Both groups were planted ear to row. Again germination of kernels with thin pericarp was poorer than those with tough pericarp. At anthesis both groups were selfed and at 23 DAP ears were harvested and frozen for further pericarp measurements.

The mean thickness in microns of cycle one selection for tough pericarp was 91.56 with a range of 74 to 120 microns (Appendix Table 6). The mean for tender selection was 87.62, ranging from 76 to 111 microns. During the second cycle, the mean for tenderness dropped to 74.21 microns with a range of 54 to 108 microns in thickness. An improvement for thin pericarp was achieved over two cycles of selection. The bite test seemed to correlate well with micrometer measurements for pericarp thickness (Appendix Table 6), however, quantitative assessment by bite is entirely impossible. Variations within ears were very small whereas variation among ears was very high.

The difference in thickness between the first and second cycle of selection was 13.41, whereas the difference among the ears of the selected parents was 54 microns in thickness. This difference is much greater than that suggested by Wolf et al. (1969) for easy separation of thin from thick pericarp by micrometer procedure. The difficulties of measuring the pericarp thickness of sugary-1 kernels were outlined by Wolf et al. (1969). At maturity, kernels are very brittle and their pericarp are extremely wrinkled. Care has been taken in order to overcome these difficulties; kernels were harvested before physiological maturity, at 23 DAP. They were either frozen with no wrinkling or dried and then rehydrated in order to stretch their pericarp. Kernels were then frozen

solid for ease of cutting the crown and the tip and for the ease of separating the pericarp from the endosperm. This method proved to be successful and the data is repeatable.

Measurements for thick pericarp were only made for one cycle of selection. A difference of 3.92 microns was found between thick and thin pericarp in the same cycle of selection (Appendix Table 6). This is quite a notable difference, considering that selection was initially made on the bite test basis.

In both first and second cycles of selection, decrease in pericarp thickness was accompanied by an increase in kernel damage (pericarp breakage) and poor germination. Similar cases of the association between thin pericarp and pericarp breakage were reported by Koehler (1957), Tatum (1942), Alberts (1927) and Meyers (1924).

Suggestions similar to those of Helm and Zuber (1972) for field corn can be made for sweet corn from this limited amount of data based on pericarp selection; selection for either thin or thick pericarp can be made by bite testing, as effectively demonstrated by Brewbaker (1968) for selection of AA8 inbred (a female parent for the H68 hybrid), but a much more rapid progress will undoubtedly be achieved with the aid of the micrometer.

It appears then that improvement in the quality of sweet and field corn (thin pericarp with more digestable material) can be made and would benefit consumers of sweet corn as well as animal feeders with field corn. Conversely, the grower may prefer corn with thick pericarp due to its association with high germination and vigorous seedlings. In the high-sucrose stocks, improvements have been made for tenderness

and high germination, and therefore suggests that improvements can be made in both directions simultaneously.

2. Pericarp, protein and dry matter of sweet and high sucrose corns during development of the kernel

Pericarp thickness, dry matter and protein synthesis of 6 genotypes in the Hawaiian Sugar background were studied during kernel development, from 14 to 42 days after pollination (DAP). H68b was used as a control. Part of these data are presented in Table 30. Pericarp thickness was determined by micrometer and protein percent of the whole kernel by micro-Kjeldahl method (A.O.A.C., 1965). Results of pericarp thickness will be discussed first followed by dry matter accumulation and protein synthesis.

Ten kernels from each genotype were selected from bulk samples and the pericarp thickness was measured at 4 positions. The mean pericarp thickness of $\underline{fl}_2\underline{sh}_2$ at 14 DAP was 61 microns and gradually increased to 101 microns at 26 DAP. For the remaining two harvesting dates of this genotype, pericarp thickness decreased. Measurement positions 1 and 2 were the germinal side and 3 and 4 were the abgerminal side. In every case, the abgerminal side was thicker than the germinal side. The $\underline{sh}_2$ genotype has shown a similar trend in pericarp development. At 14 DAP pericarp thickness was 63 microns thick and steadily increased to 99 microns at 26 DAP. For the remaining 2 harvesting dates, pericarp declined to 84 with an increase to 103 microns at the last day of harvest, 34 DAP. Again the abgerminal side of $\underline{sh}_2$ was thicker than the germinal side.

Table 30. Mean pericarp thickness in microns of 6 genotypes, 5 kernels, 4 positions, 2 sub-samples (composited from 10 ears).

$\underline{fl_2}\underline{sh_2}$

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
14	64	44	58	71	61
16	71	56	63	82	66
18	78	68	82	114	85
22	82	73	78	90	81
26	112	94	94	103	101
30	99	81	74	72	81
34	99	71	99	85	89
$\bar{x}$	78		83		

$\underline{sh_2}$

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
14	71	46	64	72	63
18	61	56	75	87	70
22	85	68	71	93	79
26	101	86	95	113	99
30	90	80	73	93	84
34	118	98	94	101	103
$\bar{x}$	80		86		

$\underline{o_2}\underline{su_1}$

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
14	68	56	66	74	66
16	49	40	57	77	58
18	--	--	--	--	--
22	85	99	96	94	93
26	103	79	75	75	83
30	98	74	86	80	84
34	83	64	90	85	80
$\bar{x}$	75		80		

$\underline{su_1}$ (HS)

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
15	53	38	53	56	50
19	54	46	67	90	64
23	101	105	118	121	111
27	97	101	97	108	102
31	92	97	90	97	94
42	93	75	76	85	82
$\bar{x}$	79		88		

$\underline{bt_1}$

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
15	88	62	93	113	89
19	90	61	90	91	83
23	99	101	122	140	116
27	85	78	90	130	96
31	84	79	95	115	93
35	75	68	80	89	78
$\bar{x}$	81		104		

$\underline{bt_2}$

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
14	65	61	66	84	68
18	90	81	76	85	81
22	78	86	97	98	90
26	63	58	74	78	68
30	91	84	87	83	86
34	80	62	72	70	71
38	57	47	59	56	54
$\bar{x}$	71		77		

The developmental pattern of $\underline{o_2}\underline{su_1}$ was somewhat similar to $\underline{fl_2}\underline{sh_2}$ and $\underline{sh_2}$ except that maximum thickness was gained at 22 DAP (93 microns) and decreased gradually to 80 microns at 34 DAP. Sugary-1 genotype (counterpart of $\underline{o_2}\underline{su_1}$) made similar gains in development, reaching its maximum thickness of 111 microns at 23 DAP. This declined to 82 microns at 42 DAP.

Pericarp thickness of both brittle-1 and brittle-2 reached its plateau of development at 23 DAP, with 116 microns and 90 microns respectively. At 35 DAP, brittle-1 had a mean pericarp thickness of 78 microns and brittle-2 (38 DAP) had 54.

All of the 6 genotypes up to this point have been in the Hawaiian Sugar background which is a variety and therefore is highly variable. Further developmental studies of pericarp thickness were conducted in the hybrid H68 which is expected to be uniform. Mean pericarp thickness of 10 kernels (2 subsamples, 5 kernels each) was taken at 8 stages of development. Data are summarized in Table 31. Maximum thickness in pericarp was reached at 18 DAP (115 microns) with a second peak of 110 microns at 22 DAP. For the next 4 harvests (24 to 28 DAP) pericarp decreased in thickness and then rose in the last two harvesting dates, 30 and 32 DAP. The differential thickness in pericarp between the two sides, germinal and abgerminal, was universal for all 6 genotypes. The largest difference recorded for the Hawaiian Sugar varieties was 23 microns (brittle-1) with 28 microns for H68.

Dry matter and protein percent of the 6 genotypes, at different stages of development, are given in Table 32. Accumulation of dry matter for all the high-sucrose corns (brittle-1, brittle-2, $\underline{fl_2}\underline{sh_2}$ and

Table 31. Mean pericarp thickness in microns of hybrid H68b at 8 stages of development.

H68

Harvest Dates	Positions 1	2	3	4	$\bar{x}$
18	102	106	114	138	115
20	95	94	108	131	107
22	97	96	111	135	110
24	90	87	112	129	104
26	88	85	114	128	104
28	85	84	109	130	102
30	96	94	106	119	104
32	93	88	114	136	108
$\bar{x}$	93		121		

Table 32. Dry matter and protein % of 6 genotypes at different stages of kernel development.

fl_2sh_2

Harvest Dates	Dry matter %	Protein %
14	16	14.00
16	19	14.00
18	24	13.00
22	25	12.60
26	26	12.50
30	29	13.00
34	30	13.00

sh_2

Harvest Dates	Dry matter %	Protein %
14	16	15.19
18	24	13.50
22	25	12.00
26	26	11.38
30	29	12.19
34	34	13.75

o_2su_1

Harvest Dates	Dry matter %	Protein %
14	24	14.50
16	27	13.56
18	29	11.69
22	33	11.38
26	37	12.56
30	43	10.93
34	44	11.50

su_1(HS)

Harvest Dates	Dry matter %	Protein %
15	20	15.19
19	28	12.69
23	33	12.37
27	38	11.31
31	40	11.50
42	52	11.43

bt_1

Harvest Dates	Dry matter %	Protein %
15	19	14.40
19	23	15.00
23	25	12.22
27	28	12.40
31	29	11.25
35	31	13.52

bt_2

Harvest Dates	Dry matter %	Protein %
14	17	13.00
18	23	13.20
22	25	11.01
26	26	13.20
30	27	12.03
34	28	12.50
38	30	15.05

$\underline{sh}_2$) followed similar patterns of development. A plateau for dry matter accumulation was reached about 26 DAP. For the sweet corn genotypes, $\underline{su}_1$ and $\underline{o}_2\underline{su}_1$, increases in dry matter were almost linear up to 30 DAP.

In June 1974 data were again collected for the dry matter contents of brittle-2 harvested at 7 stages of development. At each harvest, 500 grams of fresh weight (in duplicate) were used to obtain dry matter percent. For the seven harvests (18, 20, 22, 24, 26, 28 and 34 DAP) dry matter content was 24%, 25%, 27%, 29%, 30%, 32% and 35% respectively.

In general the protein percent of all high-sucrose corns was slightly higher than the sweet corn genotypes at any given stage of development. Rate of protein synthesis appeared to be constant throughout the stages of development for both sweet and high-sucrose corns.

SUMMARY

Corn (*zea mays* L.) lines, which carry the defective endosperm mutants brittle-1 (bt_1), brittle-2 (bt_2) and shrunken-2 (sh_2) and are referred to as high-sucrose corns, are known for poor germination and field viability. Successful improvement of viability in a Hawaiian sh_2 composite by 3 cycles of two different schemes of recurrent selection and a Hawaiian bt_2 composite by 2 cycles of one scheme of recurrent selection is demonstrated in the experiments. The use of a modified ear to row procedure proved to be effective in selection of genotypically superior individuals. Exposure of semi-lethal genes (such as sugary-1) and the sh_2 background is suggested to influence viability.

The large improvement in germination percentage after the first cycle of selection in the bt_2 and sh_2 populations is probably associated with an increase in the frequency of a small number of important genes. The subsequent slow progress of cycle-2 is presumably associated with further small changes in the frequency of the major genes and with slow increases in the frequency of minor genes. Two cycles of selection for low germination in the Hawaiian sh_2 population was ineffective.

The effect of harvesting date and storage on the tenderness, sweetness and flavor of several high-sucrose corns was examined. Harvests 18 vs 23 days after pollination were statistically non-significant. Storage for one week at 58°F made conventional sweet corn essentially unpalatable to the judges, who ranked it as badly as field corn following storage. In contrast bt_2 high-sucrose stock showed no major loss of sweetness or flavor. It is nonetheless certain that the

$\underline{bt}_2$ genotypes have shown superior quality retention in all evaluations. Sweet corn and field corn were ranked significantly poorer than high-sucrose corns on all trials.

A potential new product which has emerged as a result of these organoleptic experiments is a "freeze dried" high-sucrose corn. Freeze-dried corn is puffed, smells like fresh harvested corn, and is crisp.

All double mutant combinations involving opaque-2 ($\underline{o}_2$) and floury-2 ($\underline{fl}_2$), with $\underline{sh}_2$, $\underline{su}_1$, $\underline{bt}_1$ and $\underline{bt}_2$ were isolated in the Hawaiian sugar, CM104, and AA8 backgrounds. The $\underline{su}_1$, $\underline{sh}_2$, $\underline{bt}_1$ and $\underline{bt}_2$ genes were epistatic to both $\underline{o}_2$ and $\underline{fl}_2$ genes. The $\underline{fl}_2$ gene in the CM104 background behaves as a complete dominant gene. The crossing procedure for the establishment of the double mutant genotypes is presented.

Six diets ($\underline{++}$, $\underline{o}_2+$, $+\underline{su}_1$, $\underline{o}_2\underline{su}_1$, $\underline{sh}_2+$ and $\underline{sh}_2\underline{fl}_2$ genotypes) of corn were fed to rats and voles as the sole source of carbohydrate and protein supplemented only with vitamins and minerals. The protein efficiency ratio indicates higher nutritional values for high-sucrose corn than for sweet and field high-lysine corns. The $\underline{fl}_2\underline{sh}_2$ double mutant was significantly superior in nutritional value compared to the $\underline{sh}_2+$ single mutant. An additive effect in nutritional value of the $\underline{fl}_2$ background is demonstrated in this investigation.

The effectiveness of recurrent selection as a breeding procedure for improving pericarp thickness in sweet corn was studied. Selection was effective in both increasing and decreasing the pericarp thickness. Decrease in pericarp thickness was always accompanied with a decrease in germination percentage in sweet corn.

Pericarp thickness, dry matter accumulation and protein synthesis of 6 genotypes ($\underline{fl_2}\underline{sh_2}$, $\underline{sh_2}$, $\underline{o_2}\underline{su_1}$, $\underline{su_1}$, $\underline{bt_1}$ and $\underline{bt_2}$) in Hawaiian Sugar background were studied during kernel development from 14 to 42 days after pollination (DAP). The developmental pattern for pericarp thickness of the six genotypes was very similar. Pericarp was thinnest at 14 DAP and gradually got thicker, reaching its maximum thickness between 23 and 26 DAP. The abgerminal side of the pericarp was thicker than the germinal side for all six genotypes.

Dry matter accumulation for all the high-sucrose corns ($\underline{bt_1}$, $\underline{bt_2}$, $\underline{fl_2}\underline{sh_2}$ and $\underline{sh_2}$) followed similar patterns of development. A plateau for dry matter accumulation was reached at about 26 DAP. For sweet corn genotypes $\underline{su_1}$ and $\underline{o_2}\underline{su_1}$, increase in dry matter was almost linear up to 30 DAP.

In general the protein percent of all high-sucrose corns was higher than sweet corn genotypes at any given stage of development. Rate of protein synthesis was constant throughout the stages of development for both sweet and high-sucrose corn.

Appendix Table 1. Average dry matter %, protein % and pericarp thickness of 4 genotypes in 2 backgrounds.

18 DAP

Seedstocks	Dry matter %		Protein %		Pericarp thickness in microns	
	HS	CM104	HS	CM104	HS	CM104
$\underline{bt}_2$	20	31	14.50	11.68	68	148
$\underline{bt}_1$	29	26	10.68	14.00	98	126
$\underline{sh}_2$	25	28	14.43	10.21	104	124
$\underline{su}_1$	33	34	10.68	15.60	104	154
H68	32		11.37		94	

23 DAP

Seedstocks	HS	CM104	HS	CM104	HS	CM104
$\underline{bt}_2$	30	32	14.00	13.68	125	145
$\underline{bt}_1$	29	28	11.68	13.00	83	115
$\underline{sh}_2$	32	33	14.00	10.70	121	164
$\underline{su}_1$	40	38	11.68	14.30	106	128
H68	39		11.87		113	

Appendix Table 2. Total scores of 2 reps for tenderness, sweetness and flavor.

Tenderness — Harvested 18 DAP

	No storage										Stored 7 days									
	Judges										Judges									
Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	2	6	4	5	8	7	6	5	7	2	5	6	7	9	9	8	9	7	7	10
2	4	7	8	8	9	8	7	8	6	5	6	6	8	9	9	8	6	4	7	8
3	3	8	9	8	6	6	7	9	8	6	3	7	6	5	10	10	6	7	7	8
4	4	9	4	4	5	9	3	3	3	3	7	8	3	5	8	9	8	6	6	4
5	8	7	5	6	6	8	4	2	4	4	7	7	4	4	7	7	7	5	5	4
6	8	8	7	4	4	8	4	3	4	3	4	5	7	6	9	7	8	6	6	5
7	7	7	7	6	7	10	4	3	6	3	7	6	6	6	9	7	6	5	6	5
8	6	7	5	4	5	5	5	4	4	2	7	8	5	5	8	9	8	10	7	5
9	9	7	10	10	10	10	6	10	9	10	9	9	10	8	9	8	5	4	9	10

Sweetness

Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	10	7	8	7	9	9	8	7	9	8	10	8	10	9	10	10	10	10	9	10
2	6	9	7	9	8	9	7	7	8	8	8	8	9	8	10	10	6	8	10	10
3	8	7	9	9	8	9	8	8	9	8	10	10	9	5	10	10	9	10	10	9
4	3	4	3	2	5	3	6	4	4	2	3	7	5	4	8	6	7	9	6	6
5	2	6	4	3	4	3	2	5	5	2	3	4	6	3	8	7	7	4	5	3
6	4	7	5	2	6	4	4	4	6	4	5	6	8	9	9	10	10	10	9	9
7	4	7	4	6	6	10	4	5	7	2	4	7	9	3	10	6	5	7	9	6
8	2	4	5	2	5	6	5	6	5	3	5	8	8	4	10	8	10	10	8	7
9	8	8	10	8	10	10	10	10	10	10	9	9	10	9	10	10	10	10	10	10

Flavor

Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	5	8	7	8	9	9	7	6	9	7	8	8	9	10	10	10	10	8	9	10
2	3	7	6	8	8	9	8	7	8	7	6	7	8	8	10	8	4	8	9	10
3	3	7	8	8	8	7	8	9	10	5	9	9	8	6	10	10	10	10	9	9
4	4	7	3	3	5	6	4	7	4	2	4	8	4	5	8	7	7	10	6	8
5	7	9	2	5	5	5	5	7	4	3	5	5	6	3	7	7	7	5	5	4
6	5	6	3	2	5	4	3	5	6	3	5	5	9	4	9	7	10	10	9	8
7	6	8	3	5	6	8	7	4	6	2	3	7	9	7	9	10	8	8	8	7
8	4	5	4	4	5	5	4	6	5	2	4	8	8	5	10	8	10	10	7	8
9	9	7	10	8	10	10	10	10	7	10	8	9	10	9	10	9	9	9	10	10

Seedstock: 1(H68), 2(HS), 3(o_2su_1 COMP 1e), 4(bt_1 COMP 2e), 5(bt_2 COMP 1e), 6(sh_2 Syn 2g), 7(sh_2 COMP 2g), 8(fl_2sh_2 COMP 1b) and 9(bt_2 x su_1).

Appendix Table 3. Total scores of 2 reps for tenderness, sweetness and flavor.

Tenderness — Harvested 23 DAP

	No storage										Stored 7 days									
	Judges										Judges									
Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	3	5	3	4	5	8	5	3	7	4	7	7	7	4	9	7	7	7	7	8
2	7	6	9	6	10	9	7	6	7	8	10	7	8	4	10	8	5	8	7	9
3	7	8	8	6	8	10	8	6	7	10	8	7	7	5	10	7	6	10	6	10
4	4	8	4	3	6	7	6	5	6	5	8	6	5	6	9	8	5	7	6	6
5	8	7	8	8	7	4	6	5	4	3	7	6	5	5	10	8	7	7	6	8
6	8	7	8	3	5	8	3	3	5	3	6	6	8	6	8	7	5	6	8	4
7	7	8	5	7	8	7	6	8	6	6	5	9	8	8	10	9	6	6	7	8
8	7	9	10	8	10	10	9	10	9	10	5	8	8	8	10	8	5	8	7	6
9	7	9	10	8	10	10	9	10	9	10	7	9	9	7	9	7	6	6	7	8

Sweetness

Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	9	6	9	5	8	9	9	9	10	6	7	9	10	8	10	10	10	10	10	10
2	8	7	10	9	9	10	7	6	8	7	8	9	10	10	10	10	10	10	10	10
3	10	7	9	8	9	10	9	6	8	7	8	9	10	9	10	10	10	10	10	10
4	3	5	3	3	5	3	4	7	4	4	7	4	7	8	7	7	5	7	9	7
5	4	6	7	4	4	6	4	3	4	2	3	5	3	5	5	3	3	6	7	6
6	3	5	8	2	5	4	3	5	5	3	6	4	7	7	8	10	6	9	9	3
7	5	9	6	7	7	7	5	7	8	3	5	8	7	8	9	10	7	7	9	10
8	7	5	6	5	5	5	2	3	6	3	5	5	7	7	8	7	8	7	8	7
9	10	9	10	10	10	10	10	10	10	10	7	9	10	10	10	10	10	10	10	10

Flavor

Seedstock	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	6	5	9	6	8	9	7	9	8	6	5	9	9	6	10	10	9	10	10	10
2	6	8	10	8	8	9	5	5	8	8	7	8	9	9	10	10	7	10	10	10
3	8	7	9	8	10	10	7	5	9	7	8	8	9	7	10	10	8	10	10	10
4	6	6	3	6	5	8	5	6	6	4	8	6	5	7	8	6	5	7	9	4
5	7	7	7	8	5	8	5	5	5	2	4	4	3	7	7	6	5	7	7	5
6	5	6	8	6	7	8	3	4	4	4	5	6	7	8	8	9	4	8	9	3
7	3	9	5	8	7	6	6	6	5	4	3	7	8	8	10	10	5	7	8	10
8	4	7	6	6	7	5	2	4	5	7	5	5	7	6	9	7	5	8	7	7
9	6	8	10	10	10	10	10	10	10	10	6	8	10	9	10	10	10	9	10	9

Appendix Table 4. Total scores of 2 reps for tenderness, sweetness and flavor.

Tenderness — Harvested 23 DAP

Seedstock	Judges 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	6	6	6	7	9	7	5	8	7	6	7	7	6	10	7	5	7	4	3	10
2	6	9	6	8	3	10	6	9	8	8	10	8	5	7	9	6	7	6	5	7
3	9	8	7	7	6	6	6	4	4	7	8	6	5	3	8	5	4	6	5	8
4	6	5	7	8	10	8	4	5	9	5	6	6	5	4	7	5	2	2	5	6
5	6	8	6	8	7	7	4	4	3	6	5	3	4	5	5	4	4	4	4	5
6	7	10	7	7	6	6	6	4	6	8	5	6	2	6	7	6	4	5	5	9
7	3	6	6	7	5	3	5	3	5	8	5	6	5	4	6	3	3	4	5	4
8	5	6	6	6	4	3	5	4	4	9	8	7	8	6	6	6	3	4	5	7

Sweetness

Seedstock	Judges 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	9	9	6	7	8	8	7	8	9	7	7	10	5	9	9	9	9	4	10	10
2	10	9	7	9	7	10	8	9	10	8	9	9	6	9	10	9	9	6	9	9
3	7	5	4	7	7	7	4	5	6	8	7	7	5	6	9	10	5	6	5	7
4	6	6	2	5	5	6	2	4	4	4	4	2	3	5	6	3	2	2	2	5
5	7	8	2	5	5	6	3	2	2	4	4	3	3	4	4	4	5	5	4	4
6	8	6	4	5	6	7	4	3	4	7	5	3	2	3	7	6	4	3	7	8
7	5	5	5	6	5	7	3	2	5	7	5	6	3	3	5	5	7	4	4	6
8	6	7	5	6	6	5	3	3	9	9	8	8	5	7	7	8	7	5	7	6

Flavor

Seedstock	Judges 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	6	9	8	7	9	8	6	8	8	7	8	10	6	9	9	8	8	4	9	10
2	10	8	8	8	7	9	6	8	10	9	10	8	7	9	10	8	9	7	8	8
3	7	6	6	7	7	6	5	4	6	10	8	7	7	4	9	9	5	6	6	9
4	6	8	4	4	8	6	4	2	3	4	6	5	4	3	6	3	2	3	2	8
5	7	8	4	4	7	6	4	2	2	4	5	4	3	4	5	5	3	5	4	6
6	6	7	6	4	8	8	5	2	3	6	6	6	5	3	8	6	3	4	4	9
7	4	6	5	5	6	5	3	2	3	7	5	7	4	4	5	5	5	4	4	6
8	6	6	5	3	6	4	5	2	8	8	8	8	8	7	7	8	6	5	6	7

Seedstock: 1(H68), 2(HS), 3(o_2su_1 COMP 1e), 4(bt_1 COMP 2e), 5(bt_2 COMP 1e), 6(sh_2 Syn 2g), 7(sh_2 COMP 2g), 8(fl_2sh_2 COMP 1b).

Appendix Table 5. Average of 2 reps for per cent moisture absorbed in 4 days (at 60% relative humidity and 72°F) and colors of freeze-dried kernels of 5 genotypes with and without cooking.

HS		18 DAP moisture	18 DAP color**	23 DAP moisture	23 DAP color
	U*	8.8	Yellow group (YG11D)	10.4	Yellow group (YG4D)
$\underline{su}_1$	C	8.0	Yellow group (YG8C)	5.9	Yellow group (YG8B)
	U	8.5	Yellow orange (YO14D)	6.2	Yellow orange (YO16C)
$\underline{bt}_1$	C	7.3	Yellow group (YG11A)	6.7	Yellow orange (YO15C)
	U	8.7	Yellow group (YG11B)		
$\underline{bt}_2$	C	6.6	Yellow group (YG11A)	5.0	Yellow group (YG13C)
	U	8.8	Yellow group (YG8C)	6.6	Yellow group (YG18A)
$\underline{sh}_2$	C	7.6	Yellow group (YG10A)	6.0	Yellow orange (YO14B)
H68	U	9.6	Yellow group (YG11D)	7.8	Yellow group (YG5D)
	C	6.9	Yellow group (YG8C)	7.3	Yellow group (YG6C)

U*=Unblanched; C=blanched in boiling water for 10 minutes.
**=Colors follow Royal Horticultural Society Colour Chart.

Appendix Table 6. Raw data of pericarp thickness in microns of two cycles of selection for thin pericarp and one cycle for thick pericarp.

	C-1 Tender			C-1 Tough			C-2 Tender			C-2 Tender (cont.)			
#	I	II	Avg.	I	II	Avg.	I	II	Avg.	#	I	II	Avg.
1	74	79	76	104	106	105	66	67	67	33	76	70	73
2	87	91	89	77	81	79	87	93	90	34	60	57	58
3	81	74	78	90	91	90	64	60	62	35	83	82	82
4	91	88	90	118	121	120	62	65	64	36	78	77	78
5	85	80	82	96	93	95	78	70	74	37	74	74	74
6	71	74	72	118	114	116	79	85	82	38	81	86	84
7	70	69	70	83	78	80	76	78	77	39	82	85	84
8	76	82	79	97	99	98	75	74	74	40	61	62	62
9	76	80	78	95	99	97	63	59	61	41	86	85	86
10	98	95	96	100	95	98	74	78	76	42	60	66	63
11	74	77	76	72	70	71	51	52	52	43	87	93	90
12	113	107	110	111	101	106	73	73	73	44	62	62	62
13	101	100	100	106	92	99	54	56	55	45	79	79	79
14	123	117	120	101	105	103	81	76	78	46	84	83	84
15	112	110	111	107	105	106	66	67	66	47	98	100	99
16	92	98	95	113	110	112	73	74	74	48	73	73	73
17	102	86	94	85	84	85	97	100	98	49	72	69	70
18	73	70	72	100	100	100	74	72	73	50	78	76	77
19	92	92	92	73	78	76	84	80	82	51	80	81	80
20	77	81	79	100	103	102	92	102	97	52	60	61	60
21	95	90	92	67	66	66	83	80	82	53	91	95	93
22	74	70	72	72	67	70	91	90	90	54	71	68	70
23	88	87	88	100	99	100	77	72	74	55	59	61	60
24	84	77	80	78	74	76	106	109	108	56	67	65	66
25	94	104	99	71	77	74	55	52	54	57	70	68	69
26				74	74	74	73	75	74	58	59	59	59
27				88	84	86	90	90	90				
28				73	76	74	69	70	70				
29				82	83	82	61	61	61				
30				100	91	96	60	54	57				
31				107	104	106	71	74	72				
32				91	89	90	62	65	64				
$\bar{x}$ =	88.12	87.12	87.62	92.16	90.91	91.54	74.12	74.29	74.21				
σ_2 =	14.33	13.21		15.00	14.32		11.99	13.16					
σ^2 =	205.3	174.4		225.1	205.1		143.7	173.3					
t =	0.2566			0.3409			0.07273						
range	76 to 111			74-120			54-108						

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